THE CENTER FOR
FOOD SAFETY

Docket No. EPA-HQ-OPP-2012-0841
EPA Registration Number 524-582 (new use registration)
PP 2F8067 (petition for establishment of new tolerances)
OPP Docket, Environmental Protection Agency Docket Center (EPA/DC)
Mail Code: 28221T
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Washington, DC 20460-0001

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~~21 September 2012~~ **January 18, 2013**

Comments to EPA on:

Notice of Receipt of Application to Register New Use of Dicamba on Monsanto's Dicamba- and Glufosinate-Resistant MON 88701 Cotton (FR Vol. 77, No. 244, Dec. 19, 2012: 75153-75155); and

Requests to Establish Tolerances of Dicamba and its Metabolites 5-OH Dicamba and DCSA in or on Cotton, Undelinted Seed at 3 ppm and Cotton, Gin Byproducts at 70 ppm (FR Vol. 77, No. 244, Dec. 19, 2012: 75082-75085)

Center for Food Safety, Science Comments

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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 (e.g. Benbrook 2009a). Full citations are included at the end.

I. The impact of the requested registration on herbicide use

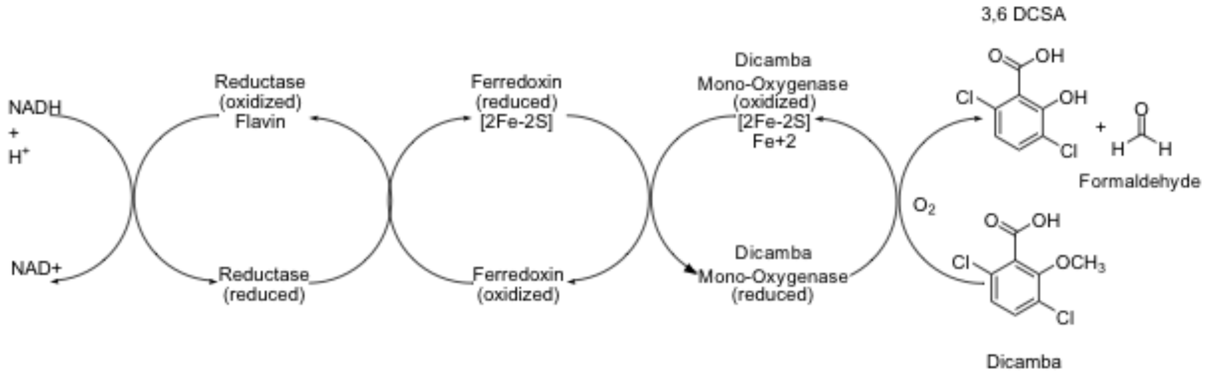
Summary of herbicide use

The requested registration would permit the use of dicamba herbicide on Monsanto's MON 87701 cotton, which is genetically engineered to withstand direct application of high rates of dicamba and glufosinate without risk of crop injury. Like many other herbicide-resistance genes used or envisioned for herbicide-resistant crops, the dicamba-resistance gene in MON 87701 is derived from a soil bacterium that was originally intended for bioremediation purposes. Public sector research originally intended to ameliorate pesticide pollution has been "repurposed" by Monsanto and other pesticide-biotech firms to facilitate vastly increased use. At present, dicamba is little used in American agriculture, and hardly at all in cotton production, with residual activity and crop injury from drift the major deterrents to wider use. MON 87701 would essentially eliminate biological constraints on use of this herbicide in cotton. In a patent application for MON 87701, Monsanto scientists propose and assert patent claims to use of up to 16 lbs./acre dicamba per season. Given widespread adoption and anticipated use rates, MON 87701 would lead to a three- to nearly six-fold increase in the amount of dicamba used in American agriculture as a whole. Monsanto's planned introduction of dicamba-resistant soybeans, corn and canola would increase dicamba use much more. Monsanto plans to market MON 87701 stacked with glyphosate resistance. Increased dicamba use is unlikely to displace much if any of the glyphosate that currently dominates weed control in cotton, meaning that overall herbicide use will rise sharply as well.

A. Introduction

The Monsanto Company seeks registration of the diglycolamine salt of dicamba herbicide for use on MON 87701 cotton, an event genetically engineered to withstand direct application of high rates of dicamba (3,6-dichloro-2-methoxybenzoic acid) and glufosinate. Monsanto also requests to establish tolerances in 40 CFR part 180 for residues of dicamba and its metabolites 3,6-dichloro-5-hydroxy-o-anisic acid (5–OH dicamba) and 3,6-dichloro-2-hydroxybenzoic acid (DCSA), in or on cotton, undelinted seed at 3 ppm and cotton, gin byproducts at 70 ppm. The following comments address both requests.

Dicamba resistance[1] is conferred by an enzyme, dicamba mono-oxygenase (DMO) that demethylates dicamba to form the non-phytotoxic 3,6-dichlorosalicylic acid (DCSA) as well as formaldhyde, as depicted below.



To generate MON 87701, Monsanto transformed cotton plants (variety name Coker 130) with a gene (*dmo*) derived from *Stenotrophomonas maltophili*a that encodes DMO (Brinker et al. 2012, 0037, 0078). A *dmo* gene with dicamba demethylating activity was initially purified from a dicamba-resistant strain of *S. maltophilia* (DI-6) that was found in soil and water samples obtained from storm water retention ponds at a dicamba manufacturing plant in Beaumont, Texas (Krueger et al. 1989[2]). In this paper, Krueger and colleagues envisioned the use of this *S. maltophilia* strain as follows:

> "Organisms capable of degrading dicamba and/or its 3,5-isomer … may be useful for facilitating the rapid dissipation of both isomers from the environment" (p. 534).

In the presence of this organism: "…dicamba is metabolized to ***compounds that are less of an environmental concern***. Complete mineralization of dicamba would result in reduced environmental exposure to degradation products and reduced potential for leaching of dicamba or its metabolites to groundwater" (p. 538, emphasis added).

Chakraborty et al. (2005), who isolated and purified DMO from this same organism,[3] also make passing reference to the function of such bacteria as "useful in the bioremediation of

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[1] Monsanto follows the biotechnology industry practice of misidentifying its crop as "dicamba-tolerant." The Weed Science Society of America has officially defined crops of this sort as "herbicide-resistant," while "herbicide-tolerance" refers to a natural species attribute. See WSSA (1998). For two of many examples of consistent, standard scientific usage of the term "herbicide-resistant" in reference to crops with this attribute, see Kruger et al. (2010a) and the following book: Nandula, VK (ed.). *Glyphosate Resistance in Crops and Weeds: History, Development, and Management*, John Wiley & Sons, Inc. 2010. EPA is urged to follow standard scientific usage and to refer to Monsanto's crop correctly as "dicamba-resistant" rather than "dicamba-tolerant" in all regulatory documents.

[2] Krueger et al. (1989) identified the source bacterium as a Pseudomonas species. In 1993, it was reclassified as *S. maltophilia*. See http://www.uptodate.com/contents/stenotrophomonas-maltophilia.

[3] Which they incorrectly named *Pseudomonas maltophilia*, apparently unaware of the 1993 reclassification to *S. maltophilia* noted in the previous footnote. Interestingly, most papers regarding *dmo* engineered into

harmful pollutants" (p. 20). Paradoxically, in the same paper, they note that DMO "has recently been utilized to develop transgenic plants that are tolerant to dicamba levels that are 10 to 20 times higher than the typical field application rate" (p. 27).

This discussion highlights a general phenomenon. There was a spate of research undertaken in the 1980s and 1990s to isolate or engineer microorganisms for bioremediation of pesticides and other toxic compounds in soils and water (e.g. at toxic waste dumps). Much of this research was funded by the public sector with the goal of reducing human and environmental exposure to toxic compounds, for instance by the Swiss government (Zipper et al. 1999) and the EPA (Short et al. 1991). To the best of our knowledge, none of these well-intentioned research efforts bore fruit in terms of successful bioremediation applications.

However, knowledge gained in this research has more recently been applied by pesticide companies and their university collaborators for the precisely contrary purpose of fostering much greater use of herbicides, which of course leads to greater pollution of the environment rather than bioremediation. For instance, Monsanto derived the EPSPS gene/enzyme utilized in most glyphosate-resistant crops from the CP4 strain of *Agrobacterium* originally isolated from the grounds of its Louisiana glyphosate manufacturing plant (Charles 2001, pp. 68-69). *Sphingobium herbicidovorans*, originally researched as a potential candidate for bioremediation of phenoxyalkanoic acid herbicides (Kohler 1999), has been utilized by Dow Chemical Co. to genetically engineer a corn variety resistant to phenoxy auxin broadleaf and AOPP ("fops") grass herbicides (Wright et al. 2010).

In fact, it has now become abundantly clear that the major focus of pesticide-biotechnology industry R&D efforts is to exploit the herbicide resistance that has evolved in soil bacteria from past use of their products to facilitate many-fold higher rates of application to plants engineered with the resistance-conferring microbial genes. Twelve of 16 GE crops awaiting deregulation by the U.S. Dept. of Agriculture are resistant to one to three herbicides each.[4] DuPont-Pioneer scientists have sketched out the industry-wide strategy of engineering multiple herbicide-resistant crops to be utilized in combination with premix formulations of the corresponding herbicides (Green et al. 2007). This same paper compiles a list of transgenes that await deployment in herbicide-resistant crops, most derived from microbes, and presumably soil microbes (see Table 1 below).

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dicamba-resistant plants make the same mistaken identification of the source organism. For one of many examples, se: Behrens MR et al. (2007).

[4] See http://www.aphis.usda.gov/biotechnology/petitions_table_pending.shtml, last visited Jan. 10, 2013.

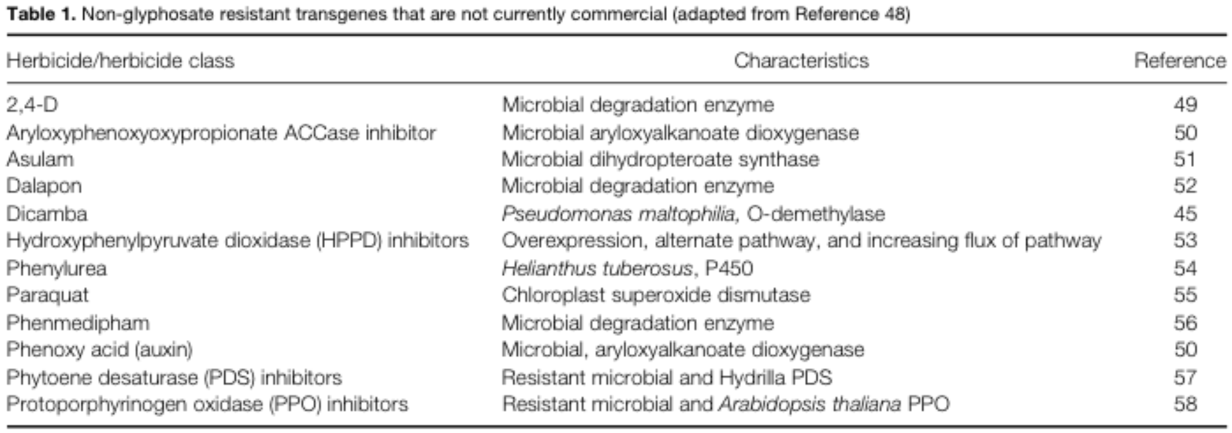

Table 1. Non-glyphosate resistant transgenes that are not currently commercial (adapted from Reference 48)

Herbicide/herbicide class	Characteristics	Reference
2,4-D	Microbial degradation enzyme	49
Aryloxyphenoxyoxypropionate ACCase inhibitor	Microbial aryloxyalkanoate dioxygenase	50
Asulam	Microbial dihydropteroate synthase	51
Dalapon	Microbial degradation enzyme	52
Dicamba	*Pseudomonas maltophilia*, O-demethylase	45
Hydroxyphenylpyruvate dioxidase (HPPD) inhibitors	Overexpression, alternate pathway, and increasing flux of pathway	53
Phenylurea	*Helianthus tuberosus*, P450	54
Paraquat	Chloroplast superoxide dismutase	55
Phenmedipham	Microbial degradation enzyme	56
Phenoxy acid (auxin)	Microbial, aryloxyalkanoate dioxygenase	50
Phytoene desaturase (PDS) inhibitors	Resistant microbial and Hydrilla PDS	57
Protoporphyrinogen oxidase (PPO) inhibitors	Resistant microbial and *Arabidopsis thaliana* PPO	58

From: Green et al. (2007).

Thus, the pesticide-biotech industry is guiding American agriculture into an era of much increased use of and dependence on pesticides, contrary to widespread misconceptions on this point.

Herbicide-resistant (HR) crops are weed control systems involving one or more applications of the HR crop-associated herbicide(s). Dow describes its 2,4-D-resistant corn and soybeans as the "Enlist Weed Control System"[5], with the brand name "Enlist" referring to both the HR trait and Dow's proprietary 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..."[6] Likewise, Monsanto speaks of integrating dicamba-resistant soybean event MON 87708 "into the Roundup Ready soybean system" (Monsanto 2010 at 211). MON 87701 will also be offered in varieties stacked with the Roundup Ready trait. As discussed further below, the intended effect of marketing the HR crop and corresponding herbicide(s) as a packaged "system" is to foster an entirely unsustainable approach to weed control that leads directly to rapid evolution of weed resistance and other adverse impacts.

B. Dicamba a little used herbicide

Dicamba is used very little in American agriculture. According to figures from the agricultural consulting firm AgroTrak, as reported by Monsanto, agricultural use of dicamba has ranged from 2.7 to 9.4 million lbs./year from 1990 to 2008 (Monsanto 2010 at 198). Dicamba thus represents just 0.5-1.6% of all herbicides applied in U.S. agriculture during this period.[7] AgroTrak data also show relatively little acreage treated with dicamba,

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[5] From: "Dow AgroSciences Petition (09-23301p) for Determination of Nonregulated Status of Herbicide-Toleran DAS-40278-9 Corn, Zea mays, Event DAS-40278-9: Draft Environmental Assessment, October 2011, USDA APHIS Docket No. DAS (2011a) as cited on APHIS–2010–0103 pp. 52, 131.

[6] 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.

[7] Total agricultural herbicide use ranged from 498-583 million lbs. from 1990-2007 (EPA Pesticide Use 2011).

ranging from just 17.4 to 36.3 million acres (Id.), or roughly 4-8% of total U.S. cropland during this period.[8]

Registered uses of dicamba include asparagus, barley, corn, cotton, fallow cropland, hay, oats, proso millet, pasture, rangeland, sorghum, soybean, sugarcane and wheat (BASF 2010).

Agricultural use of dicamba has declined over the past 15 years, peaking in 1994 at 9.4 million lbs., and declining to less than 30% of that level by 2008 (2.7 million lbs.). These figures from AgroTrak agree reasonably well with EPA estimates of dicamba's agricultural use. Acreage treated has fallen by roughly half, from 36.3 to 20.2 million acres. In 2007, dicamba was applied to just 5% of American cropland.

Dicamba is both an effective and inexpensive broadleaf herbicide. It controls a wide range of annual, biennial, perennial and woody weeds. Treatment with a typical dose of 1 pt./acre (0.25 lb/acre) costs just $3.50 to $6.44, making dicamba one of the cheaper herbicides on the market.[9] This raises the dual questions of why dicamba has never been widely used, and further why its use has declined steeply over the past 15 years.

Two basic reasons have to do with crop sensitivity to injury from dicamba. First, dicamba's residual activity necessitates considerable waiting intervals between application and planting, which makes preplant use inconvenient or impractical for many farmers. For instance, BASF's Clarity label prescribes waiting intervals of 15 days per 8 ounces/acre (=0.25 lb./acre) east of the Mississippi, and 22 days per 8 ounces/acre west of the Mississippi, for barley, oats, wheat and other monocots (BASF 2010). Second, dicamba is highly prone to cause off-target damage to nearby crops via particle and vapor drift. This is likely a factor influencing farmers in switching from dicamba to sulfonylurea herbicides in wheat, and to new broadleaf herbicides in corn (Monsanto 2010 at 197). This is discussed further below.

C. Dicamba use in cotton past and present

Dicamba is little used in cotton. Because of the crop's great sensitivity to injury from this herbicide, usage is limited to preplant and perhaps preharvest applications. Because of dicamba's residual activity, an interval of 21 days is required between application and planting, assuming minimal rainfall (or the irrigation equivalent) of 1 inch. Dicamba is not to be applied preplant to cotton west of the Rocky Mountains, or in areas with average annual rainfall of less than 25 inches (BASF 2010). According to BASF's label for Clarity, the diglycolamine salt of dicamba, the maximum single application rate for cotton is 8 ounces or 0.25 lb./acre (BASF 2010). The label also provides for a maximum in-crop

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[8] Based on USDA Agricultural Census data from 1992 to 2007, showing 406 to 435 million acres of "total cropland" during this period. Since a major use of dicamba is on rangeland, which is not included in "total cropland," these percentages may be slight overestimates.

[9] Based on price data for Banvel and Clarity in U. of Tenn. (2011).

rate/acre/year of 0.25 lb. Since dicamba is injurious to growing cotton, any in-crop application would presumably be pre-harvest, when yield would not be adversely affected.

USDA's Agricultural Chemical Usage reports[10] show virtually no dicamba applied to cotton in 1995 or 2000. Reports for 2003, 2005, 2007 and 2010 show increasing use, on 0.5%, 2.5%, 7.5% and 8.5% of Program State cotton acreage, respectively.[11] Over these same four years, the average rate/acre/year remained relatively constant, between 0.20 and 0.22 lbs.,[12] with an average of just one application for most forms of dicamba in most of these years. The total amount of dicamba applied to all U.S. upland cotton in these same years was 20,000, 81,000, 174,000 and 199,000 pounds.[13] 2010 is the last year USDA NASS surveyed pesticide use on cotton.

D. Projection of dicamba use with registration of dicamba for MON 87701 cotton

EPA registration of dicamba for MON 87701, together with USDA deregulation of the event, would likely lead to a substantial increase in dicamba use on cotton and on other crops. Important factors that must be considered in projecting the magnitude of this increased use include the rates of dicamba applied to MON 87701 and the extent of MON 87701 adoption, and the prevalence of dicamba-resistant vs. dicamba-sensitive crops in areas where MON 87701 is grown. We discuss each of these factors, and then present a projection of dicamba use.

1) Dicamba rates applied to MON 87701

i. Biological constraints

CFS is not aware of experiments that establish the rate of dicamba that MON 87701 can withstand. However, experiments with other crops genetically engineered to produce the DMO enzyme strongly suggest that MON 87701 essentially eliminates the risk of crop injury that has been the major constraint on more widespread use of this herbicide in cotton (and other crops).

Behrens et al. (2007) report that: "Many transgenic tobacco, tomato, *Arabadopsis* and soybean plants containing a nuclear-encoded *DMO* gene were fully resistant to treatments with dicamba at or above 5.8 kg/ha [5.2 lbs/acre]. This demonstrates that the *DMO* gene, present even in a single copy and expressed at relatively moderate rates (Table S2) is capable of decreasing the sensitivity of dicot plants to applications of dicamba by at least a

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[10] 2010 data accessible at http://www.nass.usda.gov/Data_and_Statistics/Pre-Defined_Queries/2010_Corn_Upland_Cotton_Fall_Potatoes/index.asp. For earlier years, see: http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1560.

[11] Figures represent the sum of % acreage treated for all forms of dicamba used, with 0.5% assumed for those forms of dicamba denoted by an asterisk (<0.5% acreage).

[12] Figures represent weighted averages for all forms of dicamba reported by USDA NASS in each year.

[13] These figures are normalized from Program State acreage surveyed by USDA NASS, which varies from year to year, to total upland cotton acreage. For instance, USDA NASS lists 168,000 lbs. of dicamba (total of four forms) applied to Program State acres in 2010, when Program State acres represented 84.5% of total cotton acreage: 168,000/0.845 = 199,000 lbs.

factor of 5000." It is reasonable to assume that MON 87701 would exhibit a similarly high level of resistance to dicamba.

In a recent patent application entitled *Cotton Transgenic Event MON 88701 and Methods of Use Thereof* (Brinker et al. 2012), Monsanto scientists report the results of field trials with MON 88701 involving 11 different treatment regimes consisting of dicamba and/or glufosinate, applied in four post-emergence treatments, as summarized in Table 2 below (from section 0088 of the patent application):

1) 4 treatments consisted of alternating applications of dicamba and glufosinate, starting with either one or the other, at 0.5 or 1 lb./acre per application;
2) 3 treatments consisted of tank-mix applications of either 0.25, 0.5 or 1.0 lb./acre of each herbicide (for 2, 4 and 8 lbs/acre/season total);
3) 2 treatments involved only dicamba, applied at either 0.5 or 1.0 lb/acre, for 2 to 4 lbs./acre per season; and
4) 2 treatments involve only glufosinate, applied at either 0.5 or 1.0 lb/acre.

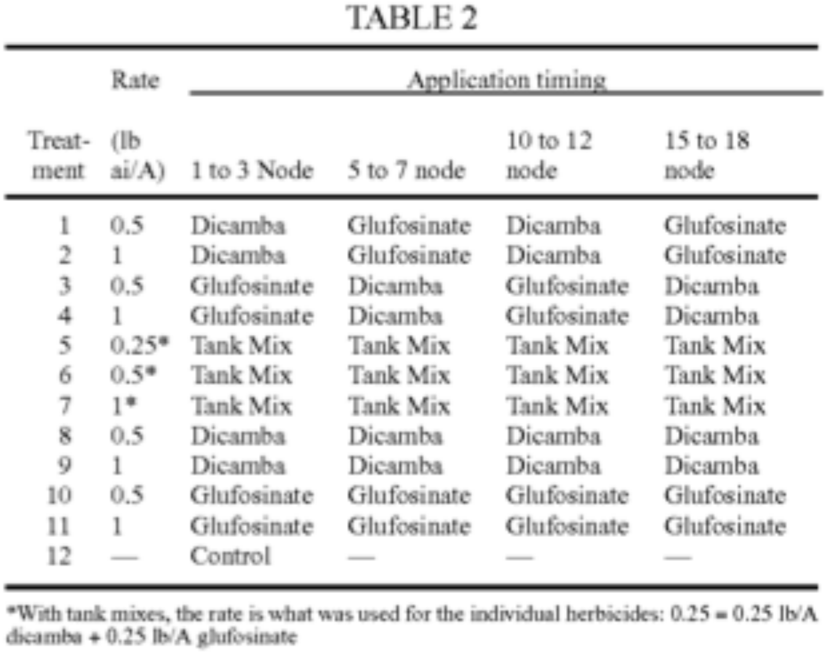

TABLE 2

	Rate	Application timing			
Treatment	(lb ai/A)	1 to 3 Node	5 to 7 node	10 to 12 node	15 to 18 node
1	0.5	Dicamba	Glufosinate	Dicamba	Glufosinate
2	1	Dicamba	Glufosinate	Dicamba	Glufosinate
3	0.5	Glufosinate	Dicamba	Glufosinate	Dicamba
4	1	Glufosinate	Dicamba	Glufosinate	Dicamba
5	0.25*	Tank Mix	Tank Mix	Tank Mix	Tank Mix
6	0.5*	Tank Mix	Tank Mix	Tank Mix	Tank Mix
7	1*	Tank Mix	Tank Mix	Tank Mix	Tank Mix
8	0.5	Dicamba	Dicamba	Dicamba	Dicamba
9	1	Dicamba	Dicamba	Dicamba	Dicamba
10	0.5	Glufosinate	Glufosinate	Glufosinate	Glufosinate
11	1	Glufosinate	Glufosinate	Glufosinate	Glufosinate
12	—	Control	—	—	—

*With tank mixes, the rate is what was used for the individual herbicides: 0.25 = 0.25 lb/A dicamba + 0.25 lb/A glufosinate

Monsanto reports that only the tankmix application involving 1.0 lb./acre each of dicamba + glufosinate (for 8 lbs./acre total herbicide over the season) resulted in a slight yield drag [Brinker et al. 2012, 0089, Figure 6]. However, the patent application elsewhere suggests that dicamba could be applied at much higher rates without risk of crop injury, consistent with the findings of Behrens et al. (2007) cited above for other DMO-producing transgenic plants.

"A herbicidally effective dose of dicamba for use in the field should consist of a range from about 0.005 pounds per acre to about 16 pounds per acre of dicamba over a growing season" (Brinker et al. 2012, 0061). Even if one assumes four applications for the season, the average application would be 4 lbs./acre.

Monsanto scientists go so far as to assert several claims to such high-level use of dicamba on MON 88701 in the patent application. Claims 20 and 21, which apply to post-emergence use, read as follows:

> 20. **A method for controlling weeds in a field** comprising planting cotton plants comprising event MON 88701 in a field and applying an effective dose of dicamba or glufosinate or dicamba and glufosinate herbicide to control weeds in said field **without injuring said cotton plants** comprising event MON 88701.
>
> 21. The method of claim 20, wherein said effective dose of dicamba herbicide is a total of about 0.005 pounds per acre to about 16 pounds per acre of dicamba herbicide over a growing season." (emphasis added)

Claims 25 and 26, which apply to preplant use, are quite similar:

> 25. A method for controlling weeds in a field comprising applying an effective dose of dicamba or glufosinate or dicamba and glufosinate herbicide to control weeds in a field and then planting cotton plants comprising event MON 88701 in said field.
>
> 26. The method of claim 25, wherein said effective dose of dicamba herbicide is from about 0.005 to about 16 pounds per acre and said planting cotton plants comprising event MON 88701 is within 14 days of said applying the effective dose of dicamba herbicide.

In a scientific publication, one could imagine scientists testing extremely high rates merely to establish the upper limits of the herbicide-resistant crop's resistance to the pertinent herbicide. Thus, in the passage quoted above Behrens et al. (2007) do not suggest that 5.2 lbs./acre is in any way a practical dose of dicamba for field use. In contrast, Monsanto scientists present (and claim patent rights to) the use of up to 16 lbs./acre dicamba per season in practical terms as a "method for controlling weeds in a field … without injuring said cotton plants…," not merely as a biological fact about the efficacy of the DMO enzyme or the level of dicamba resistance it confers on MON 87701.

EPA should clarify with Monsanto its intent in proposing and claiming patent rights to such high-rate use of dicamba herbicide on MON 87701, rates that are surely beyond anything EPA would approve.

ii. Label rates

While EPA has not proposed a label for dicamba use on MON 87701 at this stage of the registration process, information from Monsanto provides an indication of what it might be in an abstract of a presentation at a Weed Science Society of America meeting.

> "The dicamba-glufosinate tolerant technology will allow dicamba applications of up to 1 lb. ae/a pre-emergence and multiple applications of up to 0.5 lb. ae/a each in-

> crop from crop emergence to pre-harvest with a yearly total of up to 2 lb. ae/a" (Voth et al. 2012).

This statement is of course incorrect. As discussed above, MON 87701 "dicamba-glufosinate tolerant technology will allow" much higher-rate applications of dicamba than those listed here without risk of crop injury. We take this statement to reflect instead the label rates for which Monsanto is seeking approval from EPA.

2) MON 87701 adoption

MON 87701 could well be widely adopted by farmers, for several reasons. First, Monsanto is promoting MON 87701 as a response to the epidemic of glyphosate-resistant (GR) weeds, which are particularly problematic in cotton (see herbicide-resistant weed discussion below). Most GR weeds are broadleaf plants, and so susceptible to dicamba. The two most prevalent and problematic GR weeds – Palmer amaranth and horseweed - are very prevalent in cotton fields of the south, and increasingly in cotton fields of western states like California and Arizona.

Second, dicamba use has increased significantly since 2003, from <0.5% of cotton acreage to 8.5% in 2010. This probably reflects rising preplant use as a response to glyphosate-resistant weeds. MON 87701 would eliminate the 21-day interval presently required to avoid crop injury to the emerging cotton seedling. It would also presumably increase the allowable preplant rate four-fold, from 0.25 lb/acre at present to 1 lb./acre. Both factors would make dicamba – the use of which is already on the rise in cotton – still more attractive to cotton growers battling GR weeds. MON 87701 would also presumably lift the minimum rainfall and regional restrictions on use of dicamba in cotton, expanding the range of its use west of the Rocky Mountains and into drier regions elsewhere. Perhaps the most important factor is that MON 87701 would appeal to the predilection of cotton growers accustomed to total or near total POST weed control programs with Roundup Ready cotton by permitting two or more multiple post-emergence applications of the herbicide. Finally, Voth et al.'s statement quoted above suggests that Monsanto is seeking approval for preharvest use of dicamba as well. It is unclear to what extent this would be utilized, but since preharvest applications are often made as "rescue" treatments to fields heavily infested with larger weeds, preharvest application rates tend to be higher, since higher rates are generally needed to kill dense populations of larger weeds.

A third important factor is that introduction of MON 87701 will likely be preceded by dicamba-resistant soybeans. Soybeans are extremely sensitive to injury from low-level dicamba drift, and are widely grown in cotton-growing states like Arkansas, Mississippi, North Carolina, Tennessee, Louisiana and Missouri. Cotton growers who also grow soybeans, or who have neighbors growing soybeans, might be reluctant to adopt the dicamba-intensive MON 87701 system from fear of dicamba drift harming their own or their neighbors' non-dicamba-resistant soybeans. Conversely, significant prior adoption of dicamba-resistant soybeans would erode these inhibitions, and make adoption of MON 87701 more likely.

Steve Smith, the Director of Agriculture at Red Gold, an Indiana-based tomato processor which sources tomatoes from family farmers in Indiana, Ohio and Michigan, explains a very similar dynamic with respect to dicamba use on corn being fostered by widespread adoption of dicamba-resistant soybeans:

> "The use of dicamba is not new. It has been a labeled product for use on corn for decades. It has been proven effective for many uses and is not particularly vulnerable to developing resistant strains of weeds. It is economical to apply.
>
> So many may be wondering why a product that is effective, proven, and economical is not the number one herbicide in use today. The answer is simple. Dicamba has proven itself to move off-target and cause injury and yield reductions to soybeans and so in a large sense, it is rarely used. Farmers respect their neighbors and know they are at risk of causing injury if they use dicamba, so it is not widely and routinely used in corn production. However, when soybeans become tolerant to dicamba, it is very likely that the amount of dicamba used in corn production will skyrocket when the fear of soybean injury is eliminated. As an example, when glyphosate soybeans were first introduced, there was significant injury due to drift on corn the first few years. It didn't take long for applicators and farmers to gain a higher degree of respect for the injury that could occur. But once the widespread use of glyphosate resistant corn became common, that level of caution began to erode because it didn't really matter if you drifted onto your neighbor, because their crop was also glyphosate resistant. I also predict a similar fate for dicamba use once soybeans are made tolerant. With no fear of soybean injury, the use of dicamba on corn acreage will dramatically increase, raising the overall exposure of sensitive crops to injury. Because dicamba is deadly to weeds and cheap to use, it is a sure prediction that dicamba use will increase dramatically, followed by escalating crop losses." (Smith 2010)

3) Projection of dicamba use with MON 87701

MON 87701 would facilitate season-long use of dicamba. The patent claims noted above apply to both pre-plant and post-emergence use of potentially very high rates of dicamba without risk of crop injury. The field trials reported in the patent application utilized up to 4 lbs/acre dicamba per season in four post-emergence applications. Voth et al. (2012) suggest also that post-emergence treatments would extend to the pre-harvest period, which could further increase usage.

The projection in the table below estimates dicamba use given adoption of MON 87701 on 50%, 75% or 100% of US cotton acreage, with average application rates of 50%, 75% and 100% of the presumed maximum label rate of 2 lbs./acre per season.

Projection of Dicamba Use with MON 87701

	% adoption MON 87701		
Average rate/year (lbs.)	**50%**	**75%**	**100%**
1.0	5,664,500	8,496,750	11,329,000
1.5	8,496,750	12,745,125	16,993,500
2.0	11,329,000	16,993,500	22,658,000

Based on five-year average cotton acres planted from 2008-2012 = 11,329,000 acres

Measured against 2010 cotton usage of dicamba (199,000 lbs., as discussed above), MON 87701 would lead to a 28-fold increase in dicamba use on cotton (to 5.7 million lbs.) in the most conservative scenario, which assumes just half the label rate is utilized and MON 87701 is planted on 50% of cotton acres. The scenario in which MON 87701 is planted on 75% of cotton acreage and growers apply 75% of the label rate would lead to dicamba use of 12.7 million lbs., or 64-fold more than was applied to cotton in 2010. If all cotton were MON 88701 and the full (presumed) label rate were applied, dicamba use would total 22.7 million lbs., or over 100-fold more dicamba than was used on cotton in 2010.

This range of scenarios for a second-generation herbicide-resistant cotton event appears reasonable in light of the extremely widespread adoption of and heavy use of glyphosate on first generation herbicide-resistant cotton, Monsanto's Roundup Ready. USDA AMS reports that 98% of cotton acres planted in 2011 were transgenic, the vast majority glyphosate-resistant (USDA AMS 2011).[14] USDA NASS Agricultural Chemical Usage figures for 2010 show that glyphosate was applied to 99% of cotton acres at an average annual rate of 1.66 lbs./acre (USDA NASS 2010).[15] As discussed further below, the severity of GR weeds in cotton makes high adoption of MON 87701 likely. Dicamba usage rates may initially be lower than projected, but will climb rapidly with the inevitable rapid emergence of dicamba-resistant weeds.

Agrotrak data cited above show 2.7 million lbs. of dicamba used in agriculture nationally in 2008. In the most conservative scenario above (50% adoption, ½ the label rate), MON 87701 would triple agricultural use of dicamba, from 2.7 to 8.4 million lbs. With 75% adoption and 75% of the label rate, dicamba use would rise nearly six-fold, to 15.4 million lbs., while the full presumed label rate with 100% adoption would lead to more than an 8-fold increase in agricultural dicamba use, to 25.4 million lbs. In prior comments to EPA, CFS projected the impact on dicamba use of the widespread adoption of Monsanto's MON 87708 dicamba-resistant soybeans, assuming EPA registration.

> "Granting the proposed registration would thus likely entail a 50 million lb. increase in dicamba use on soybeans, assuming a realistic adoption scenario, by 2025; and an additional, conservatively estimated 8 million lb. increase in use on non-dicamba-resistant corn, for 58 million lbs. of dicamba applied annually. This projection ignores the clear potential for greater dicamba use in wheat in areas where it is grown near soybeans. Further increases to be expected with introduction of dicamba-resistant corn and cotton would easily drive this figure to well over 100

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[14] USDA's Agricultural Marketing Service publishes annual "Cotton Varieties Planted" reports which give detailed data on the percent of US cotton acres planted to virtually all cotton varieties grown, at both state and national levels. These data are regarded by cotton experts as more reliable than the figures for GE cotton in the better known USDA ERS reports ("Adoption of Genetically Engineered Crops in the U.S.)" In the cited USDA AMS report, glyphosate-resistant varieties are denoted by suffices containing the letters "R,," "RR," "RF," "F" (Roundup Ready Flex) or "GT" (Bayer's Glytol cotton).

[15] 99% is the sum of % cotton acres treated for four forms of glyphosate; 1.66 lbs/acre/year is the weighted average rate/year of these four forms of glyphosate.

> million lbs. per year. 58 million lbs. would represent a more than 20-fold increase over current agricultural use of dicamba herbicides." (CFS 2012, p. 12)

This latter estimate is based on a projection of increased auxin and overall herbicide use with adoption of dicamba- and 2,4-D-resistant soybeans in Mortensen et al. (2012), from which the following figure is reproduced.

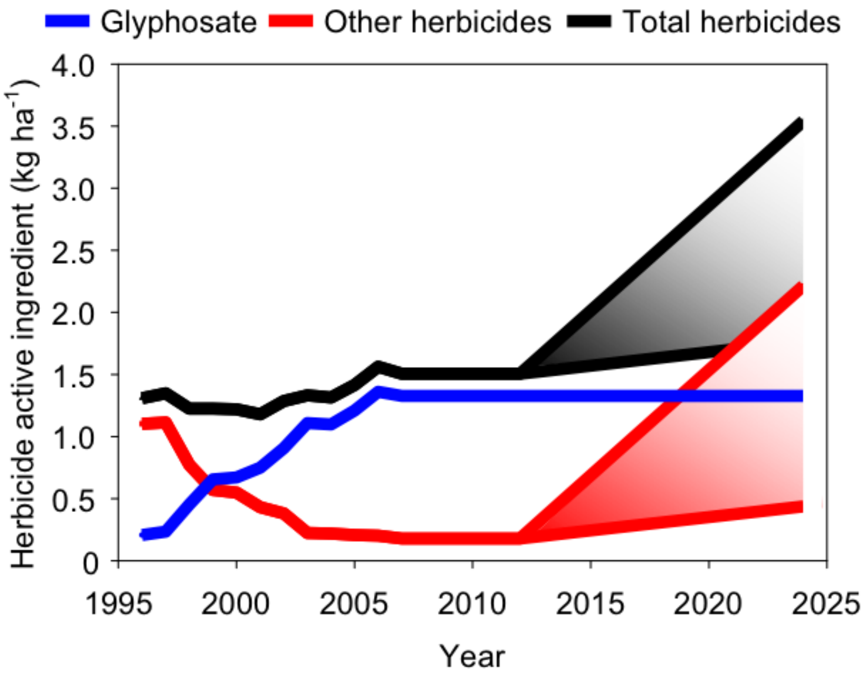
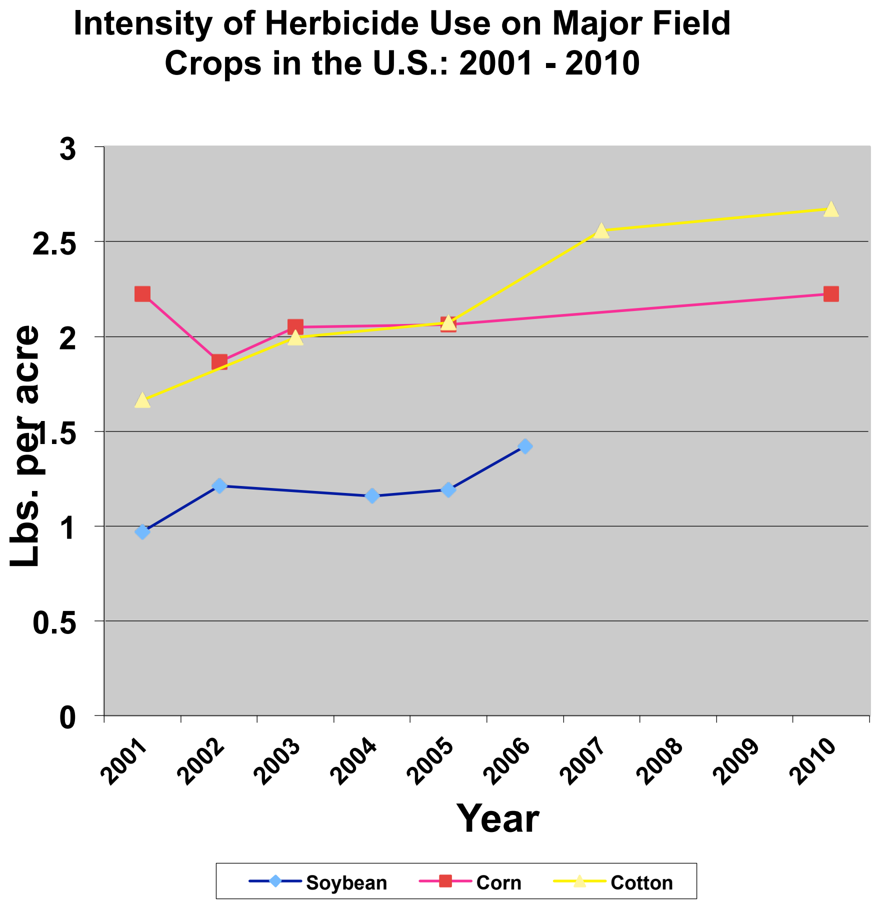


Figure 4. Total herbicide active ingredient applied to soybean in the United States. The data from 1996 to 2007 are adapted from Figure 2-1 in NRC (2010), and the projected data are based on herbicide programs described by Arnevik (2010) and Olson and Peterson (2011). To forecast herbicide rates from 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.

4) Overall herbicide use as impacted by MON 87701

It is unlikely that dicamba would displace much if any glyphosate in cotton. Monsanto will offer MON 87701 in a stacked version with glyphosate resistance (Voth et al. 2012). Because dicamba is a broadleaf herbicide, broad-spectrum glyphosate will continue to be used with MON 87701 to kill grassy weeds that are not as sensitive to dicamba. It is not clear to what extent MON 87701's glufosinate-resistance will impact dicamba use. Glufosinate-resistant cotton varieties (LibertyLink) have long been available to farmers, but little used, even as glyphosate-resistant weeds proliferated. For instance, in 2010 only 7% of cotton acres were treated with glufosinate,[16] which likely reflects LibertyLink cotton adoption. Growers long familiar with Roundup Ready technology will be inclined to continue using glyphosate at current or near-current rates, and rely primarily on dicamba to kill glyphosate-resistant weeds. Thus, both dicamba and overall herbicide use will increase sharply with adoption of MON 87701, continuing the trend of sharply rising herbicide use in cotton since 2001 illustrated in the figure below.



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

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[16] USDA NASS Agricultural Chemical Usage report for 2010, cited above.

II. The impact of the requested registration on evolution of herbicide-resistant weeds

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 more rapid evolution of resistant weeds than do other (non-HR crop) uses of the pertinent herbicide(s). This is clearly demonstrated by the history of glyphosate-resistant weeds, which have emerged almost exclusively in the Roundup Ready crop era. Weeds resistant to synthetic auxin herbicides, the class to which dicamba belongs, are already numerous, indicating that auxin-resistance is prevalent in the plant world. The requested registration would facilitate greatly increased dicamba use on weeds already resistant to glyphosate and other herbicides, leading to still more intractable, multiple herbicide-resistant weeds. Clear evidence of cross-resistance and/or tolerance to auxin herbicides among weed species exacerbates the threat. Multiple herbicide-resistant weeds lead to increased selection pressure for resistance to evolve to the ever fewer remaining effective herbicidal control options. In light of these considerations, weed scientists have recently called for mandatory stewardship practices to address the likely emergence of auxin-resistant weeds with auxin-resistant crop systems. Voluntary stewardship efforts to forestall HR weeds have failed in the past, and will not succeed with MON 87701. Because herbicide-resistant weeds, once evolved, can spread their resistance traits via cross-pollination and seed dispersal, stewardship recommendations that focus on persuading individual growers to "do the right thing" are ineffective, and risk undermining the utility of valuable herbicides for non HR crop uses. Regulation is a rational response to this "tragedy of the commons" dilemma, in which the susceptibility to weeds is the common resource rapidly being squandered. The multiple herbicide-resistance of MON 87701 cotton volunteers would make them problematic weeds, and thus impede efforts to eradicate boll weevils and imperil efforts to forestall insect resistance to Bt toxins.

A. 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 corn 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 results 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.

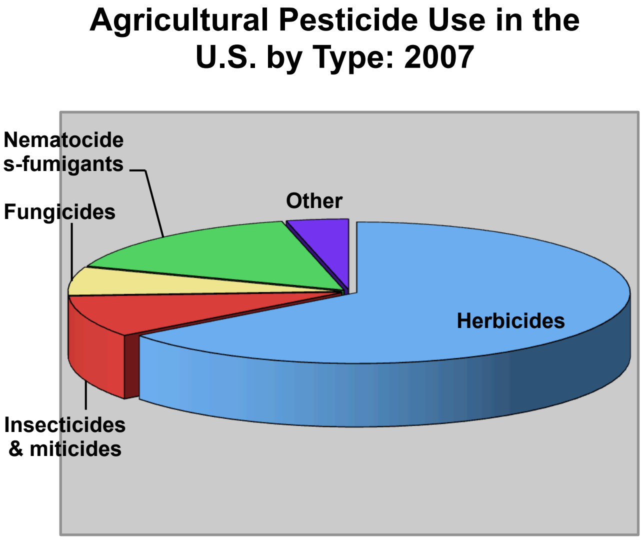
Unfortunately, with the exception of crop rotation and tillage, such techniques are little used in mainstream agriculture. This is in no way inevitable. Education and outreach by extension officers, financial incentives to adopt improved practices, and regulatory requirements are just a few of the mechanisms 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, exacerbated by the widespread adoption of herbicide-resistant crops, are becoming ever more serious.

B. 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 9/20/12).

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 and then multiple-drug 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).

Besides costing farmers economically via herbicide-resistant weeds, the chemical-intensive pest control regime of HR crop systems 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 demonstrate 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 (e.g. cyanazine) 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.

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

Herbicide-resistant (HR) crop systems such as MON 87701 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 auxin-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).

To our knowledge, Monsanto has not revealed its pricing for MON 87701 cotton seed, but it is likely to be considerably more expensive than currently available GE varieties.

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; dicamba provides control of most broadleaf weeds. Applied together or sequentially, glyphosate and dicamba would initially provide broad-spectrum control of soybean weeds, making use of other weed control measures unnecessary until the inevitable rapid evolution of auxin 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. Dicamba 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 price of the associated herbicide(s) is low, the likely scenario with MON 87701 cotton. Any price premium for a dicamba product registered for use on MON 87701 would encourage farmers to use cheaper and more drift-prone formulations, whether registered for that use or not.

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

Glyphosate-resistant crop systems have fostered later post-emergence applications than many agronomists anticipated, which increases the potential for resistant weed evolution:

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

> 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)

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 varieties) 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.

D. 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 MON 87701 cotton system. Second, the prevalence of glyphosate-resistant weeds is the motivating factor in

Monsanto's introduction and farmers' potential adoption of MON 87701 under the requested registration.

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. 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).[18] Based on acreage infested, GR weeds have emerged overwhelmingly in soybeans, cotton and cotton 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).[19]

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 Sept. of 2012, as many as 440,000 sites on up to 18.7 million acres were documented to be infested by glyphosate-resistant weeds (CFS GR Weed List 9/20/12). This astonishing

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[18] 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 cotton. See http://www.weedscience.org/Case/Case.asp?ResistID=5682.

[19] Now 14 weed species, in at least 31 states. GR weeds have been documented in four additional states since this 4/22/12 list was compiled. For South Dakota, Wisconsin and Arizona, see list at http://www.weedscience.org/Summary/UspeciesMOA.asp?lstMOAID=12&FmHRACGroup=Go. For Montana, see AgNews (2012).

proliferation of resistant weeds – an over 130-fold increase in number of sites and 8-fold increase in acreage – is portrayed in the figure at the end of this section.

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. Inclusion of this 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 an even higher estimate of GR weed-infested acreage of 60 million acres (Bomgardner 2012). Thus, actual acreage infested with glyphosate-resistant weeds is double to triple the 18.7 million acres reported by ISHRW and shown in the figure below. However, the figure does provide a reasonably accurate representation of the extremely rapid pace 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 corn with the Roundup Ready trait has increased sharply in recent years, from 20% to 72% of national corn acres from just 2004 to 2011. The increasing reliance on glyphosate associated with the growing use of RR soybean/RR corn rotations is the major factor driving 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. One recent example is the emergence of GR common waterhemp on land planted to soybeans, corn 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 kochia, 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).

E. Synthetic auxin-resistant crops and weeds

Synthetic auxin herbicides like dicamba act by mimicking plant growth hormones such as indole acetic acid. In its petition for deregulation of dicamba-resistant soybeans (MON 87708), Monsanto maintains that "there is a low potential for dicamba-resistant broadleaf weed populations to arise from the use of dicamba applied to MON 87708 integrated into the Roundup Ready soybean system," and gives the following reasons for this opinion (Monsanto 2010, p. 601).

1) Dicamba will be used together with glyphosate, with recommended use of a soil residual herbicide, and such use of multiple modes of action "is a primary way to delay the development of resistance;"
2) Resistance to auxin herbicides has developed slowly, hypothetically due to multiple sites of action within plants, suggesting that resistance is determined by multiple genes as a quantitative trait;
3) Only four broadleaf weeds have been confirmed as resistant to dicamba in the U.S., while relatively low numbers of weed species have confirmed resistance to synthetic auxin herbicides in general; and
4) Confirmed dicamba- and auxin-resistant weeds are found primarily in the West rather than in major soybean production regions, and weeds with known dicamba resistance are not major soybean weeds.

Monsanto would presumably make similar arguments with respect to MON 87701. However, there are several serious flaws in these arguments, which were persuasively rebutted by Mortensen et al. (2012). First, Monsanto's two points regarding past history of auxin- and dicamba-resistant weed emergence have little bearing on the future course of resistance with introduction of MON 87701 under the proposed registration. As explained above, use of an herbicide in the context of an HR crop system very significantly elevates the risk of resistant weed emergence relative to non-HR crop uses of the same herbicide. Monsanto officers cannot fail to understand this, given the history of glyphosate-resistant weeds with their RR crops, but apparently prefer to ignore the lesson.

However, even to the limited extent that past resistance is relevant, Monsanto is in error. The ISHRW website lists 49 biotypes[20] of 30 different weed species with resistance to synthetic auxin herbicides internationally (ISHRW SynAux Weeds 9/20/12). Of the 21herbicide modes of action to which weeds have evolved resistance, synthetic auxin-resistant weeds rank fourth in terms of number of resistant species, in the top quintile (ISHRW HR Weed Ranking 9/20/12). Contrary to Monsanto, this is a quite high number of resistant species relative to other modes of action, and it indicates that the genetic

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[20] We use the term "biotype" to refer to a single listing on the ISHRW website. For instance, four biotypes of the single species kochia have evolved auxin resistance in four different states.

predisposition to survive auxin treatment is quite prevalent in the plant world. Moreover, five new auxin-resistant biotypes and 1 new species have been recorded by ISHRW over just the past five months,[21] indicative of continuing and perhaps accelerated emergence of auxin-resistant weeds.

Nine biotypes of five different weed species have confirmed resistance to dicamba: lambsquarters (1), common hempnettle (1), kochia (4), prickly lettuce (1) and wild mustard (2) (see ISHRW SynAux Weeds Table 9/20/12 for following discussion). One other biotype highly resistant to 2,4-D also exhibits reduced sensitivity to dicamba (common waterhemp in Nebraska, discussed further below). Interestingly, four biotypes of four species have confirmed resistance to dicamba and other auxin herbicides, while one other population has multiple resistance to dicamba and several ALS inhibitors. The cross-resistance of dicamba-resistant weeds (to other auxin herbicides is troubling, because it suggests that at least some resistance mechanisms confer simultaneous resistance to multiple auxin herbicides, which reduces alternative weed control options. Indeed, even Monsanto's dicamba-resistant soybeans exhibit increased tolerance to all three phenoxy herbicides that were tested (Monsanto 2010, pp. 74-77). CFS has not found similar data for MON 88701. Many auxin-resistant weeds have not been tested for dicamba resistance, so there could be considerably more weed species and biotypes that are immune to the herbicide.

The argument that auxin-resistant weeds have developed slowly due to multiple sites of action in the plant is also speculative and likely 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. This is particularly true of auxin resistance, understanding of which continues to evolve (for a recent review, see Grossman 2010). 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: dearth of resistance from past use of glyphosate, and the molecular nuances of glyphosate's mode of action.[22] These predictions were of course disastrously wrong, but they did help quell concerns about GR weed evolution and forestall efforts to establish mandatory weed resistance management programs as Monsanto was introducing its Roundup Ready crops (Freese 2010, response #1). Interestingly, only one GR weed had been identified by the time the first RR crop was introduced in 1996 (ISHRW GR Weeds 4/22/12), in contrast to the 30 weed species with biotypes resistant to auxins 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

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[21] 45 biotypes and 29 species when CFS last recorded these data (compare ISHRW SynAux Weeds 4/22/12 to ISHRW SynAux Weeds 9/20/12).

[22] Interestingly, another reason put forward by Monsanto scientists Bradshaw and colleagues for the unlikelihood of GR weed evolution was Monsanto's past failures in multiple attempts to engineer glyphosate-resistant plants, the arrogant presumption being that Nature could certainly not accomplish what had proven so difficult for Monsanto's scientists.

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.

Monsanto's third argument, that use of both dicamba and glyphosate on MON 87708 soybeans stacked with glyphosate resistance will hinder evolution of weeds resistant to either one, also lacks merit. (The same argument would presumably be applied to MON 87701 stacked with Roundup Ready.) This argument ignores the obvious fact that the huge extent of existing GR weed populations – with many billions of individual weeds on 30 to 60 million infested acres – make it near certain that some among them will have the rare genetic mutations conferring resistance to dicamba ***as well***. Mortensen et al. (2012) provide the mathematical exposition (emphasis added):

> First, when an 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 dicamba-resistant crop systems like MON 87701 cotton will very likely foster rapid evolution of weeds resistant to dicamba and glyphosate. In those cases where the GR weed populations in dicamba-treated 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 dicamba and glyphosate.

F. 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),[23] 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 15 biotypes of weeds resistant to glyphosate and one or more other herbicide families in the U.S. (13) and Canada (2). The great majority is found on land planted to cotton, soybeans and/or corn (and in a few cases other crop or non-crop settings), where Roundup Ready crops predominate. All but one have emerged since 2005.[24]

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 dicamba 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. In a recent survey, weed scientists deemed these four weed species as most likely to evolve problematic populations of dicamba-resistant weeds (Crespo 2011).

1) 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 9/20/12[25]), up from 3.3 million acres in 16 states in February 2009 (Benbrook 2009a, p. 35). It is particularly prevalent in Tennessee, Kansas

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

[24] See individual reports listed at http://www.weedscience.org/Summary/UspeciesMOA.asp?lstMOAID=12&FmHRACGroup=Go, last visited 1/14/13.

[25] 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.

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 in California a population of horseweed's *Conyza* relative, hairy fleabane, with dual resistance to glyphosate and paraquat was recently reported to infest up to 1 million acres.[26] Ohio has glyphosate/ALS inhibitor-resistant[27] horseweed.

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. In 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 MON 87701. Yet Purdue University weed scientists have flagged horseweed as a plant 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]

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[26] See http://www.weedscience.org/Case/Case.asp?ResistID=5390.

[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).

[28] As noted above, horseweed has also evolved dual resistance to glyphosate and paraquat in Mississippi; in California, a glyphosate/paraquat-resistant biotype of the closely related *Conyza* weed hairy fleabane was recently reported to infest up to 100,000 fields on as much as 1 million acres. See http://www.weedscience.org/Case/Case.asp?ResistID=5250.

These same scientists have already founded increased tolerance to dicamba and 2,4-D in several horseweed populations, demonstrating the high potential for horseweed to evolve additional resistance to dicamba in the context of heavy postemergence use enabled by the proposed registration:

> "Population 66 expressed almost twofold greater tolerance to 2,4-D ester and approximately three- to fourfold greater tolerance to diglycolamine salt of dicamba than populations 3 and 34 (Table 1). Population 43 was more sensitive to growth regulators than population 66 but expressed slightly higher levels of tolerance to 2,4-D ester and diglycolamine salt of dicamba than populations 3 and 34 based on dry weight measurements." (Kruger et al. 2010b)

It is significant that these two populations each exhibit increased tolerance to both dicamba and 2,4-D, indicating the potential for evolution of resistance to both herbicides if either one is used (cross-resistance). In addition, the increased tolerance to dicamba of both populations was found only with the diglycolamine, but not the dimethylamine salt of dicamba, suggesting that the proposed registration might more readily lead to auxin-resistant horseweed than would other forms of dicamba.

Kruger et al. also predict that auxin herbicides will be applied later to larger horseweed plants in the context of auxin-resistant crop systems (Kruger et al. 2010a). In follow-up research, they found that larger plants are much more difficult to control with auxin herbicides:

> ***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 [dicamba and 2,4-D]. ***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 dicamba with dicamba-resistant crops, because that is precisely how growers use these crop systems, as demonstrated with the history of RR crops.

This tendency to delay application to kill larger weeds will be greatly facilitated by the high-level dicamba resistance of MON 87701, since the higher rates needed to kill larger weeds are readily tolerated by MON 87701. As discussed above in relation to RR crops, farmers delay application in order to avoid the trouble and expense of a second application, whether this is a wise tactic or not. Thus, advising growers to spray weeds when they are small will likely not be any more effective with MON 87701 than were similar recommendations made for glyphosate with Roundup Ready crops.

Cultivation of MON 87701 under the requested registration is quite likely to promote rapid evolution of horseweed resistant to dicamba and perhaps 2,4-D as well, 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 dicamba/glyphosate-resistant horseweed, since dicamba will be eliminated as an alternative control option. This would lead to further reductions in conservation tillage and increased soil erosion.

2) 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 12 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 9/20/12). 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 alone. Though a bigger problem in Corn Belt states, GR waterhemp is also emerging in cotton-growing states like Tennessee and Mississippi.

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 9/20/12 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 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 with 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, a waterhemp population highly resistant to 2,4-D and with significantly reduced sensitivity to dicamba was discovered (Bernards et al. 2012), 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 auxin-resistant crops – "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 MON 87701 cotton and other auxin-resistant 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 their concerns. 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 MON 87701 cotton. Second, this population also has significantly reduced sensitivity 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. More generally, this waterhemp example corroborates the evidence presented above regarding the prevalence of auxin-resistant biotypes with cross-resistance to several auxins (including 4 biotypes with confirmed resistance to dicamba and other auxin herbicides), as well as the findings of Kruger and colleagues regarding increased tolerance to both 2,4-D and dicamba in certain horseweed populations.

It is interesting to note that the field where this waterhemp evolved resistance to 2,4-D and increased tolerance to 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. At least in the case of this waterhemp population, this strategy apparently did not work. Annual use of three major modes of action failed to forestall resistant to one of them, casting doubt on the herbicidal onslaught approach to weed management.

Monsanto scientists discuss 2011 field trials with MON 87701 stacked with Roundup Ready Flex that involved preemergence use of a residual herbicide, dicamba or a tank mix of both, followed by various post combinations of glyphosate, dicamba, acetochlor and glufosinate (Voth et al. 2012). Best results were achieved when a pre-emergence residual herbicide was included. However, residual herbicides have become extremely unpopular with the rise of Roundup Ready crops, which have inculcated a strong preference for total post-emergence herbicide programs. Multiple HR crops like MON 87701 will reinforce this tendency, making use of residuals still less likely, especially in light of the added expense of using a third or fourth herbicide on top of expensive seed and use of two to three herbicides POST.

However, this waterhemp population suggests that the herbicidal onslaught approach suggested by Monsanto's field trials may not always be successful even if utilized. In addition, 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.

3) 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. Cotton is listed as a crop setting for GR Palmer amaranth in 10 of 18 U.S. biotypes (CFS GR Weed List 9/20/12, with one additional biotype recently reported in Arizona cotton[29]). Best known for plaguing cotton and soybean growers in Southern states, this weed is rapidly emerging in Corn Belt states like Illinois and Missouri; populations have recently been reported in Michigan (ISHRW GR Weed List 4/22/12) and Ohio (Ohio Farmer 2012). In California, a population of GR Palmer amaranth has just been reported infesting three predominantly Roundup Ready crops (alfalfa, corn, cotton) as well as

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[29] http://www.weedscience.org/Case/Case.asp?ResistID=5699.

orchards, vineyards, roadways and fencelines.[30] 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).

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 9/20/12). Palmer amaranth belongs to the same genus as common waterhemp (*Amaranthus*), and to some extent can interbreed with it. Both have considerable genetic diversity. The demonstrated ability of waterhemp to evolve resistance to auxin herbicides suggests that a similar potential likely exists in Palmer amaranth. Cotton growers with GR and multiple HR Palmer amaranth would be prime candidates to adopt MON 87701, and utilize them under the requested registration. Palmer amaranth is judged a high-risk weed for evolution of resistance to dicamba and other auxin herbicides (Crespo 2012), which would undermine the efficacy of existing, pre-emergence use of dicamba in battling this serious weed threat.

4) 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 cotton, soybeans and corn 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; a third in South Dakota (2011) infests up to 10,000 acres and was first listed in May of 2012; while a fourth infesting up to 1,000 acres in North Dakota was first listed in August of 2012. Kochia resistant to both glyphosate and ALS inhibitors was recently identified in Alberta, Canada (2012).[31] 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.

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[30] http://www.weedscience.org/Case/Case.asp?ResistID=5690.

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

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 dicamba in Montana, Idaho, North Dakota, and most recently Nebraska. The Nebraska population first emerged in corn in 2010, and Nebraska is a major soybean producing state. Nearly half of all confirmed dicamba-resistant weed populations in the world are kochia biotypes, which may suggest a genetic proclivity in this species to evolve resistance to this herbicide. The extremely rapid emergence of GR biotypes in RR crop systems (e.g. in Kansas cotton) may induce growers to adopt MON 87701 to control it; and kochia's demonstrated propensity to evolve resistance to dicamba make it a prime candidate to evolve multiple resistance to dicamba, glyphosate and other herbicides.

G. Stewardship

It is highly doubtful whether any voluntary stewardship plan for MON 87701 cotton would be effective in forestalling weed resistance to dicamba. This is because dicamba-resistant crop introduction will likely recapitulate the history with Roundup Ready crops.

One reason GR weeds are so problematic in Roundup Ready cotton is that so much cotton is grown continuously, 73% of cotton acres according to USDA (USDA ERS 2006), and the vast majority of cotton varieties offered and grown over recent years contain the Roundup Ready trait. This set up a situation where post-emergence glyphosate was the primary or even sole weed control tool used by many cotton farmers year after year, though since the emergence of GR weeds use of other herbicides has become increasingly common. It is beyond the scope of these comments to address the reasons for Roundup Ready monoculture in cotton, but it is clearly some combination of farmer choice and Monsanto's long-standing strategy of "biotech trait penetration," which involves withdrawing non-GE and especially non-RR varieties of cotton (i.e. conventional and Bt only) and other crops from the marketplace. While many cotton growers likely choose to grow RR cotton varieties continuously, others who would like to diversify have few non-RR choices (see Freese 2007, Sections 2.4 & 2.5).

However, even to the small extent that cotton is rotated (20% of cotton acres are rotated with row crops, according to USDA ERS 2006), it has largely failed to forestall GR weed emergence. Though USDA does not specify which row crops are rotated with cotton, soybeans and to a lesser extent corn are likely the major rotation partners. Soybeans are generally more prevalent than corn in major cotton-producing states, such as Arkansas, Mississippi, North Carolina, Missouri, Tennessee and Louisiana, while corn is more common in Texas and Georgia. Rotation from cotton to these crops has in most cases not offered any break from glyphosate selection pressure since these rotation partners are predominantly Roundup Ready as well. Indeed, analysis of ISHRW data by CFS shows that the great majority of GR weed infested acreage in the U.S. is on land growing either cotton and soybeans or corn, cotton and soybeans (CFS GR Weed List 9/20/12). Crop rotation has little benefit in forestalling weed resistance when all of the crops in the rotation are resistant to the same herbicide(s) and post-emergence use of this/these herbicide(s) is the chief or only weed control tool utilized.

Introduction of MON 87701 will likely be preceded by dicamba-resistant soybeans, since Monsanto has both petitioned USDA for deregulation of this crop (no petition has yet been posted for MON 87701) and applied to EPA for the corresponding registration. Monsanto also has dicamba-resistant corn and canola in its longer-term pipeline (Monsanto 2011, slide 16). Thus, there is a clear potential for widespread planting of four major dicamba-resistant crops and heavy reliance on post-emergence dicamba to control weeds in them every year, a sure recipe for weed resistance.

Monsanto is unlikely to offer much assistance in this regard, and if past history is any guide, will contribute to rather than seek to forestall evolution of dicamba-resistant weeds. As Roundup Ready crops were being introduced, Monsanto denied that glyphosate-weeds would evolve (Bradshaw et al. 1997; see also Freese 2010). Similarly today, Monsanto claims that "there is a low potential for dicamba-resistant broadleaf weed populations to arise from the use of dicamba applied to MON 87708 integrated into the Roundup Ready soybean system," and would presumably make a similar claim with regard to MON 87701. The fallacies of this and similar self-serving messages were discussed above. Denial of the problem at the outset offers little hope that Monsanto will engage in meaningful weed resistance prevention actions.

In 2003 and 2004, when GR weeds were emerging at a rapid pace in RR soybeans, Monsanto promoted "glyphosate-only" weed control programs in farm press advertisements, ads that leading weed scientists castigated as irresponsible for promoting weed resistance (Hartzler et al. 2004). These ads, emblazoned with the logo for Roundup Ready corn, were designed to encourage reluctant farmers who were already growing Roundup Reaady soybeans to grow Roundup Ready corn as well, setting the stage for massive GR weed emergence in the corn/soybean belt. Monsanto will likewise promote adoption of its stable of dicamba-resistant crops, without regard to the weed resistance risks that they pose in the aggregate.

After massive emergence of GR weeds, Monsanto began recommending that growers introduce soil residual herbicides to complement post-emergence glyphosate with Roundup Ready crops. Monsanto will likely make similar recommendations with MON 88701. Growers already paying a presumably high premium for MON 88701 seed precisely because of the ability it provides to control weeds post-emergence will thus be advised to apply glyphosate, dicamba and glufosinate as well as one or more additional (residual) herbicides, which most growers will understandably find economically infeasible as well as environmentally undesirable.

What would a responsible weed resistance management program look like? First, Monsanto would simply choose NOT to recapitulate the history of Roundup Ready crops and glyphosate-resistant weeds by introducing dicamba resistance into four major field crops. Second, it would prohibit farmers from planting dicamba-resistant crops in successive years by including a corresponding clause in its technology use agreement, following the precedent of BASF with its Clearfield wheat (see CFS RRSB 2010, pp. 23-24). Third, it would offer its most desirable varieties of cotton, soybeans and corn in less expensive conventional and Bt only-versions as well as dicamba-resistant versions, thus giving farmers the choice and incentive to rotate away from continuous post-emergence use of dicamba.

Since Monsanto will not undertake any of these measures, it is up to the USDA and EPA to impose mandatory weed resistance prevention plans as a condition of deregulation of dicamba-resistant crops, and registration of dicamba for use on them. Other potential regulatory actions are suggested in Mortensen et al. (2012).

Such plans must account for the resistance-promoting impacts of all dicamba-resistant crops, for instance when grown in rotation; and given the clear potential for cross-resistance in weeds to various auxin herbicides, they should encompass all auxin-resistant crops as a group (e.g. including Dow's 2,4-D-resistant crops). CFS is not alone in calling for such an approach. Bernards et al. (2012) have also recognized the failure of voluntary stewardship in this area, and have called for "mandatory stewardship practices" to forestall weed resistance for "soybean, cotton and corn resistant to 2,4-D and dicamba."

H. 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 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.

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 9/20/12), can travel for tens to hundreds of kilometers in the wind, which is likely an important factor in the extremely rapid spread and prevalence of GR horseweed. Hybridization among related weeds is another potential means by which resistance could be spread, for instance among weeds in the problematic *Amaranthus* genus (Gaines et al. 2012). Movement of resistant seed via waterways when heavy rainfall leads to flooding has been suggested as one explanation for the epidemic spread of glyphosate-resistant and multiple herbicide-resistant waterhemp[32] in the sugarbeet production region of Minnesota and North Dakota (Stachler et al. 2012). In Kentucky, weed scientist James Martin states that flooding has likely contributed to the spread in Kentucky of GR Palmer amaranth and waterhemp, as they were first observed in fields situated in flood plains or river bottoms along the Ohio, Green and Mississippi Rivers. These weeds are now spreading to upland fields (Pratt 2012). In Arkansas, weed scientist Ken Smith also reports that herbicide-resistant weed seeds are transported by floods, explaining why HR weeds like Palmer amaranth, waterhemp and Johnsongrass are first discovered along waterways (Bennett 2011). Smith says that flooding of the Mississippi in 2011 is sending glyphosate-resistant Palmer amaranth seed from Arkansas to Louisiana, and is introducing herbicide-resistant waterhemp from upriver to growers of his state, setting back progress made by individual farmers through implementation of on-farm HR weed prevention and control measures.

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, either from neighboring fields in the case of resistant pollen flow and local flooding, or from fields many miles distant through long-distance airborne seed transport or larger flooding events on major rivers. 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

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[32] For the recent confirmation of multiple HR waterhemp, see http://www.ag.ndsu.edu/homemoisture/cpr/weeds/herbicide-resistance-in-waterhemp-in-mn-and-nd-and-management-in-sugarbeet-corn-and-soybean-5-24-12.

less incentive for any farmer to expend the time and resources required for prevention measures. Similar spread of dicamba-resistant weeds is likely with introduction of MON 87701.

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 of some assurance that other farmers in their area will do likewise. This too suggests the need for mandatory weed resistance plans to forestall emergence of auxin-resistant weeds in the context of MON 87701 cotton and similar auxin-resistant crops.

I. MON 87701 cotton will compromise boll weevil eradication efforts and could accelerate insect resistance to Bt toxins

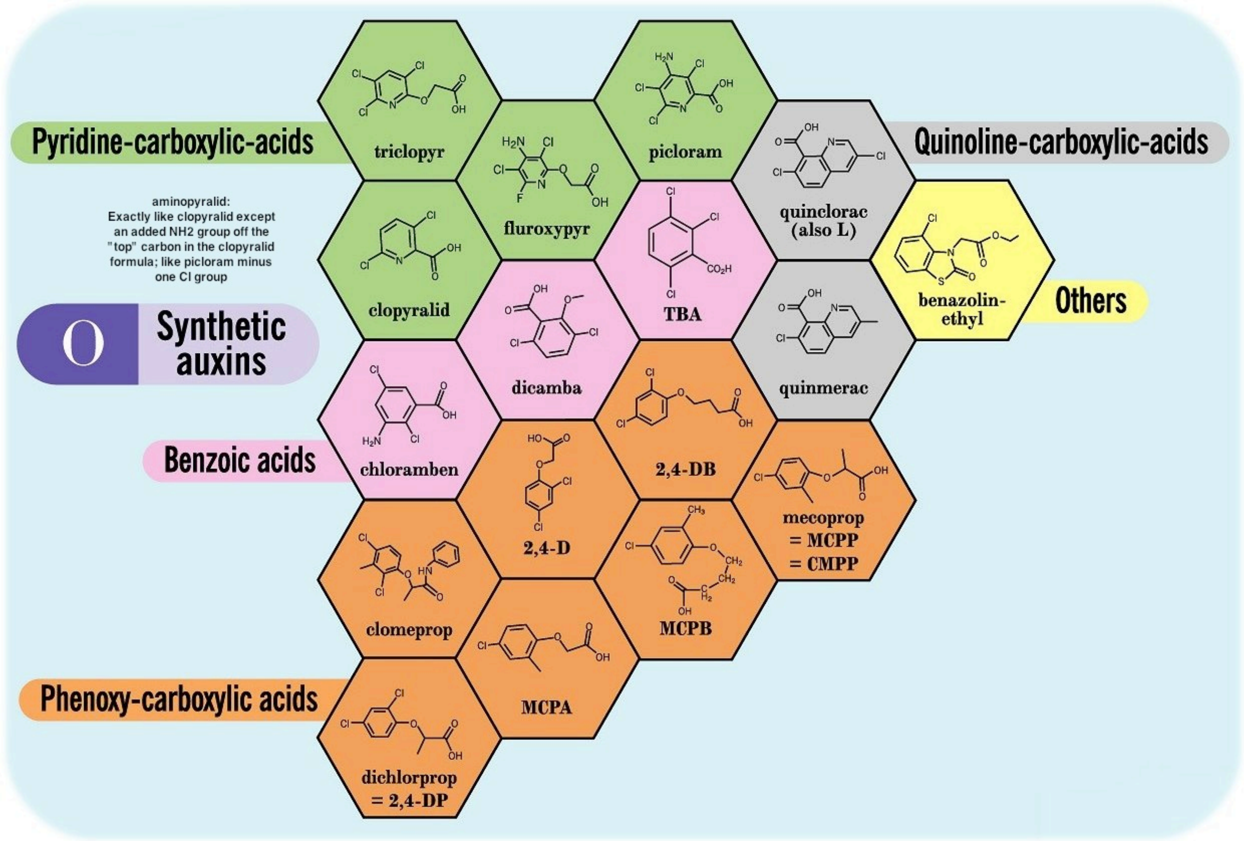
1) Weedy volunteer MON 87701 cotton and the boll weevil

Volunteer cotton can be a problematic weed in follow-on cotton or rotational crops due to high seed survival from one season to the next, and has become particularly challenging with the advent of Roundup Ready and LibertyLink technologies, as volunteers of these varieties are resistant to glyphosate and/or glufosinate (Morgan et al. undated). Volunteer cotton becomes much more difficult to control if allowed to grow past the 2-3 leaf stage (Thomson & Steckel undated), and growth from the 1-leaf to the 4-leaf stage occurs in just 10-14 days, which means a short window of opportunity for effective control (Bowman et al. 2012).

Volunteer cotton not only acts as a weed, reducing yields of crops in which it emerges, it can also harbor boll weevils. In Tennessee (Thompson & Steckel undated), Texas (Morgan et al. 2011), and perhaps other states, growers are legally required to control volunteer cotton plants in cotton or other crops as part of boll weevil eradication efforts. While 80-90% control of volunteer cotton is acceptable to prevent yield loss, in quarantine zones Texas state law requires 100% control of non-commercial (e.g. volunteer) cotton plants at the 6-8-leaf stage or larger, which is considered the size at which they become viable hosts for boll weevils (Morgan et al. 2011).

Texas agronomists are concerned that multiple herbicide-resistant cotton varieties, such as MON 87701 stacked with Roundup Ready and Dow's 2,4-D/glyphosate/glufosinate-resistant cotton, will make volunteer cotton "much more difficult to manage" and thus could impede boll weevil eradication efforts (Morgan et al. undated). Herbicide efficacy trials carried out in Texas established that: "Few herbicides are currently labeled in corn, sorghum or wheat that provide excellent control of small and larger cotton and prevent boll weevil hostable plants beyond 40 days after treatment" (Morgan et al. undated). Pre-emergence herbicides (even 2 lbs/acre atrazine) were generally ineffective, providing at best 65% control (Morgan et al. 2011).

2,4-D, dicamba, Status (a premix of dicamba and diflufenzopyr), glufosinate and glyphosate (applied at 1.125 lbs./acre) comprised the majority of effective herbicidal options for post-emergence control of volunteer cotton (Morgan et al. 2011, Table 5). These options would of course not be available for volunteers of MON87701 stacked with Roundup Ready, with the possible exception of 2,4-D. However, Monsanto has reported that its MON 87708 soybean, which like MON 87701 incorporates the DMO enzyme for dicamba resistance, exhibits increased tolerance to three phenoxy herbicides similar in structure to dicamba: 2,4-D, 2,4-DB and MCPA (Monsanto 2010, pp. 74-77, see figure below). In herbicide injury field trials, Monsanto found that each of these herbicides caused significantly less damage to MON 87708 than to control soybeans without the DMO enzyme 20 days after application (Monsanto 2010, Table C-7, pp. 317-318). While this increased tolerance to the three major phenoxy herbicides on the market today may not be sufficient to permit their use on MON 87708 to control weeds, it would make control of volunteer soybeans more difficult, and at least necessitate higher doses than are needed for non-dicamba-resistant soybean volunteers. Volunteers of MON 87701 may similarly be more difficult to control with 2,4-D, 2,4-DB and/or MCPA than volunteers of other cotton varieties, impairing the efficacy of these important control options and/or necessitating higher rates.



Source: http://www.weedscience.org/summary/HRACchem.asp.

2) MON 87701 cotton stalk destruction and the boll weevil

A related aspect of boll weevil eradication efforts is the requirement, in Texas and perhaps other states, to destroy cotton stalks soon after harvest to prevent boll weevil survival into the winter (for this discussion, see Smith 2012). 2,4-D has been the most effective and widely used herbicide for this task. According to Texas cotton expert Galyon Morgan, "triple-stacked herbicide tolerant cotton varieties will complicate cotton stalk destruction." In the anticipation of introduction of auxin-resistant cotton varieties, Morgan is testing alternate herbicides for stalk destruction, including 2,4-DP, MCPP, Weedmaster (a premix of dicamba and 2,4-D), Harmony Extra (a premix of thifensulfuron and tribenuron), Distinct (a premix of dicamba and another auxin herbicide, diflufenzopyr) and Clarity (dicamba).

MON 87701's dicamba-resistance would disqualify Clarity. Its dicamba resistance and presumably increased tolerance to 2,4-D (see discussion above) would greatly reduce the efficacy of Weedmaster and Distinct. As illustrated in the figure above, two other options being tested by Morgan are phenoxy herbicides very similar in structure to 2,4-D: 2,4-DP (dichlorprop) and MCPP (mecoprop) (see figure above). Monsanto did not include these lesser-used phenoxy herbicides in the MON 88708 herbicide injury trials mentioned above, so it is not clear if MON 88708 and MON 87701 would have increased tolerance to them as well. If so, essentially all of the herbicidal options noted above would be disqualified or impaired for use in destroying MON 87701 cotton stalks.

Another option for control of volunteer cotton and presumably for destruction of cotton stalks is tillage. Bowman et al. (2012) recommend tillage for volunteer cotton that exceed the 4-leaf stage, while Morgan et al. (2011) regard tillage as an effective means to reduce volunteer cotton competition with crops, but inadequate for boll weevil eradication efforts.

Thus, introduction of MON 87701 (as well as 2,4-D-resistant cotton) could pose serious obstacles to boll weevil eradication efforts in Texas and other cotton-growing states, and at the very least increase the toxicity and cost of these efforts by necessitating application of higher rates and tank mixes of several herbicides in cotton, or alternately increase the use of tillage and associated soil erosion.

3) MON 87701 volunteers and insect resistance management for Bt toxins

This is not the first time agronomists have found that herbicide-resistant crop volunteers impair efforts to control damaging insect pests. Krupke et al. (2009) examined volunteers of stacked glyphosate-resistant/insect-resistant corn emerging in follow-on glyphosate-resistant soybeans. They found that 65% of the volunteers tested positive for CryBb1 (corn rootworm toxin), and that 60% tested positive for both glyphosate-resistance and CryBb1. Surprisingly, CryBb1-positive volunteers exhibited the same degree of root damage from larval rootworm feeding as volunteers that tested negative for the CryBb1 rootworm toxin. They hypothesize that these volunteers may produce less CryBb1 toxin due to deficient nitrogen in soybean fields that are not amended with this nutrient. Exposure of corn rootworm to low-levels of Cry3Bb1 in corn volunteers will likely accelerate evolution of resistance, undermining insect resistance management efforts.

Corn volunteers have become an increasingly problematic weed in their research area (Indiana) and throughout the Corn Belt because glyphosate-only weed control programs with Roundup Ready soybeans fail to control glyphosate-resistant/Bt corn volunteers in common soy/corn rotations. Krupke et al. conclude that "weedy volunteer corn plants stacked with GR [glyphosate-resistance] and Bt traits may accelerate the development of Bt-resistant WCR [western corn rootworm] populations, circumventing the current [Bt insect-resistance] management plans."

The great majority of U.S. cotton grown today is stacked with both herbicide- and insect-resistance (USDA AMS 2011). MON 87701/RR cotton will also be offered primarily in varieties stacked with Bt; its resistance to three herbicides and presumably increased tolerance to phenoxy herbicides will make cotton volunteers much more difficult to control and hence prevalent. If MON 87701/RR cotton volunteers produce lower levels of Bt toxins, as is apparently true of stacked corn volunteers, they could accelerate evolution of resistance in pests of the bollworm complex.

4) Pollen- or Seed-Mediated Gene Flow Among HR Cotton Plants

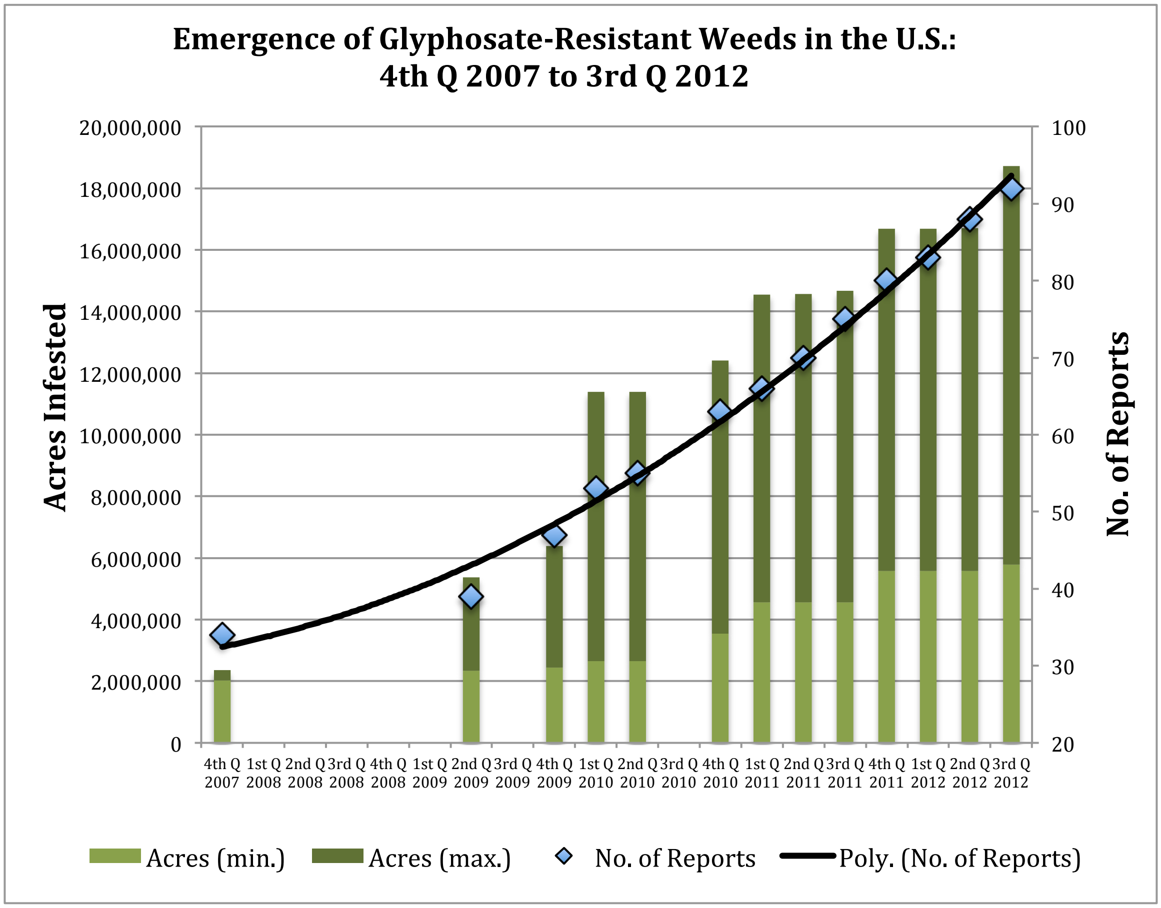
Transgene flow from a genetically engineered crop into commercial seed lots has been documented in corn, canola, soybeans and cotton (Mellon & Rissler 2004, Heuberger et al. 2010). In a study conducted in Arizona, Heuberger et al. (2010) assessed the relative contribution of seed- versus pollen-mediated gene flow as factors in the contamination of non-Bt cotton with a Bt gene common in commercial cotton varieties (Cry1Ac). Six non-Bt seedlots were planted in 15 fields in a cotton-growing area of Arizona. Prior testing of the seedlots and additional tests of plants in the field revealed that two seedlots were contaminated with Bt seeds, at levels of 20% and 0.5%, indicative of seed-mediated gene flow from planting error in production of those seedlots. Pollen-mediated gene flow was also observed in 10 of the 15 fields, based on positive tests for the Cry1Ac protein in seeds of plants on the edges of, and 20 meters into, the non-Bt production fields. The percentage of outcrossed (Cry1Ac-containing) seed ranged from 0.13% to 0.71% (Table 1). Outcrossing was best correlated with the area of Bt cotton fields within 750 meters of the seed production field, and was also positively associated with the abundance of honeybees.

While transgene flow has been studied primarily in the context of "contamination" of conventional (non-genetically-engineered) varieties, it is obvious that GE varieties may also exchange genes. The study by Heuberger et al. described above indicates that transgene flow can result in considerable contamination, either through seed-mediated gene flow due to planting error, or through insect-vectored pollen-mediated gene flow.

MON 87701 seed could thus acquire additional herbicide-resistance traits through seed- or pollen-mediated gene flow from other HR cotton cultivars growing near MON 87701 fields, either through planting error or cross-pollination. Even low contamination rates of MON 87701 seed with the transgene conferring 2,4-D resistance from Dow's Enlist cotton (for instance) would exacerbate the problems discussed above with regard to boll weevil eradication efforts and management of insect resistance to Bt toxins.

Adventitous presence or volunteers of triple-resistant MON 87701/RR cotton with increased tolerance to phenoxy herbicides, and still more the potential for quad-stack MON 87701 cotton resistant to 2,4-D as well due to gene flow, reduces the array of herbicidal options for control of such plants, and thereby accelerates evolution of resistance to those few herbicides that remain effective and feasible in the particular crop setting.

The discussion above demonstrates that registration of dicamba for use on MON 87701 and deregulation of the event may well lead to exacerbation of several serious insect pest problems. Since volunteers can in general serve as vectors of insect pests and pathogens, and act as bridges over time to maintain pest/pathogen populations in the rotational off-year, the increased prevalence of MON 87701 volunteers may well pose other, yet unsuspected pest problems. In general, regulatory decisions on HR crops and their associated herbicides pose risks of a sort with which EPA is not normally confronted when making herbicide registration decisions. Regulatory assessment of and decisions on multiple herbicide-resistant crops and the herbicides used with them must be greatly expanded to encompass these largely novel and serious concerns.



<u>Legend</u>: This chart plots data on glyphosate-resistant weeds in the U.S. compiled from the International Survey of Herbicide-Resistant Weeds (ISHRW) as of September 20, 2012. See CFS GR Weed List 9/20/12 for the data upon which this chart is based. The ISHRW lists reports of confirmed herbicide-resistant weeds submitted by weed scientists.[33] 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 13 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, 12/31/11, 3/28/12, 7/2/12 and 9/20/12 – corresponding to the 13 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. The figures shown here are very conservative, because ISHRW is a voluntary reporting system and many GR weed populations are never reported, or if reported are often not updated to account for expansion. ISHRW organizer Dr. Ian Heap concedes that these figures are "way too low," and in August 2012 estimated that 40 million acres were infested with a GR weed (30 million if overlapping acres infested with more than one GR weed are counted just once) (see Heap 2012). As noted in the text, Dow estimates 60 million GR weed-infested acres. This suggests that GR weed prevalence is roughly twice to three times the upper-bound estimates shown here. Even so, this graph provides a sense of the rapid course of GR weed emergence in the U.S.

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

III. The requested registration would sharply increase non-target crop and plant injury from dicamba particle and vapor drift episodes

Dicamba use under the requested registration would likely lead to a considerable increase in injury to non-target crops and wild plants, for reasons related to features of HR crop systems in general and to the properties of dicamba in particular. As discussed above, HR crop systems facilitate postemergence weed control programs in which the herbicide is generally applied much later in the season, when neighboring crops have leafed out and are more susceptible to drift injury. High-level resistance to the herbicide facilitates use of higher rates without risk of crop injury, which increases the drift "dose" that non-target crops and plants receive, as well as the range of drift at levels that can damage plants. When adoption of a crop resistant to a particular herbicide or herbicide combination reaches a certain critical mass, some farmers feel compelled to adopt it or another crop with the same resistance traits for defensive reasons; and others become less cautious in drift prevention measures, on the assumption that most of their neighbors are growing crops with the same herbicide-resistance trait(s).

Roundup Ready crops illustrate the problem, as explained well by Steve Smith in the passage quoted above. Although glyphosate is not a drift-prone or volatile herbicide, two surveys of state pesticide regulators have found that glyphosate consistently ranked second, behind only 2,4-D, in drift-related crop injury episodes (AAPCO 1999, 2005). These surveys were conducted from 1996-1998 and 2002-2004 during the years of rapid Roundup Ready crop adoption and associated sharp rise in postemergence glyphosate use. In Arkansas, an important factor encouraging Roundup Ready corn adoption was to defend against drift from glyphosate use on Roundup Ready soybeans and cotton. According to Ford L. Baldwin, of Arkansas-based Practical Weed Consultants, Inc.:

> "A lot of growers planted Roundup Ready corn in the beginning out of self defense. I looked at enough glyphosate drift on conventional corn to understand why." (Baldwin 2010)[34]

In Mississippi, agronomist Trey Koger observes that the threat of Roundup misapplication and drift from Roundup Ready fields is a major disincentive for farmers who would otherwise be inclined to grow conventional soybeans (Bennett 2009). Steve Smith, Director of Agriculture for Red Gold, an Indiana-based tomato processor, reports that tomato farmers based in Indiana, Ohio and Michigan who supply his firm experienced over $1 million in losses over a four-year period from glyphosate drift emanating from Roundup Ready crop fields (Smith 2010). In controlled experiments, Kruger et al. (2012) have confirmed that glyphosate causes substantial yield reductions to tomatoes at low, drift-level doses.

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[34] Baldwin goes on to note that "defensive adopters" of RR corn eventually transitioned to from conventional herbicides to a glyphosate-based program, exacerbating GR weed evolution.

Many of the same agronomic factors that make glyphosate drift problematic will obtain with MON 87701 and other dicamba-resistant crops. Use of dicamba herbicide would increase dramatically with MON 87701, to an estimated 5.7 to 12.7 million lbs. Adoption of MON 87701 would be facilitated if previously introduced MON 87708 soybeans are being widely grown. The latter would drive perhaps another 50 million lbs. of dicamba use on soybeans. The prevalence of dicamba-resistant cotton and soybeans would erode corn growers' concerns about drift injury, and increase their use of dicamba as well, perhaps by 8 million lbs. The great majority of these dicamba applications would likely be later in the season, increasing drift damage. The acreage of sensitive crops within damaging drift range increases with expanding dicamba-resistant crop acreage

Properties of dicamba make it a much greater threat than glyphosate. Like most auxin herbicides, dicamba has very broad-spectrum activity on broadleaf plants, which include cotton and soybeans as well as nearly all vegetables and fruits. Auxin herbicides are also very potent, dicamba more so than 2,4-D, such that very low drift-level doses can cause considerable damage. Finally, while most herbicides pose a drift threat only during their initial application, dicamba is extremely volatile, and is known to volatilize from plant surfaces days after the initial application to move off-target and cause damage to crops and wild plants at considerable distances.

Behrens and Lueschen (1979) report that post-emergence dicamba sprays used on 250,000 ha of corn in Minnesota in 1974 resulted in 68 reports of dicamba drift effects on soybeans. In contrast, post-emergence use of 2,4-D on 800,000 ha hectares of corn yielded just seven reports. This suggests that pound for pound, dicamba is much more prone to cause drift-related crop damage than 2,4-D. This finding is supported by the AAPCO surveys referenced above. In these surveys, dicamba consistently ranked third among all herbicides in drift-related crop injury episodes, behind only 2,4-D and glyphosate, despite its extremely limited use. In the latter survey period (2002-2004), just 4-5 million lbs./year of dicamba were used agriculturally in the entire country (Monsanto 2010 at 198). In the same period (2003), roughly 30 million lbs. of 2,4-D and 130 million lbs. of glyphosate were applied annually (EPA Pesticide Use 2011), 7-fold to 30-fold more, respectively.

In a series of field and glasshouse experiments, Behrens and Lueschen (1979) established that dicamba, volatilizing after application to corn, caused symptoms on soybean plants placed up to 60 meters downwind of the treated corn; that dicamba volatilizing from treated corn could be detected via effects on soybeans for three days after the application; and that dicamba volatilization was enhanced by higher temperatures and lower humidity, and extinguished by rainfall.

Interestingly, this team determined that dicamba acid and various salt forms had widely varying volatilization rates from glass surfaces, and that the vapors of more volatile salts (after application to corn) caused much greater damage to nearby soybeans in closed jars than did the less volatile salts. However, in field experiments, these differences largely disappeared. That is, less volatile salts applied to corn vaporized to damage downwind soybeans almost as much as the highly volatile (e.g. dimethylamine) salts. The diglycolamine salt being proposed for registration is apparently less volatile than the widely used dimethylamine salt. However, this may not translate into lesser injury to crops from volatilization. Even if the diglycolamine salt of dicamba is less prone to vapor drift, there is no way EPA or Monsanto would be able to prevent use of more drift-prone formulations such as the dimethylamine salt.

In tests involving the diglycolamine salt of dicamba, Andersen et al. (2004) simulated dicamba drift injury by directly treating soybeans with 5.6 to 56 g a.e./ha dicamba (1% to 10% of the label rate for corn). These treatments reduced soybean yields by 14% to 93%. Andersen et al. found greater soybean injury in the drier of the two years of their experiment, in line with the findings of Behren and Lueschen that lower humidity enhanced volatilization, and rainfall extinguished it. Finally, it was found that dicamba applied in a mixture with crop oil concentrate, which enhances absorption of the active ingredient by crop tissues, resulted in slightly higher levels of injury. This highlights the importance of considering dicamba's activity in the forms in which it is actually used by farmers.

Kelly et al. (2005) examined the impact of low-level dicamba in combination with other post-emergent herbicides on soybeans, to simulate the effect of dicamba vapor drift in a realistic soybean production setting. Similar to Andersen et al., this team found yield reductions from application of 5.6 g a.e./ha dicamba (1% the label rate for corn) either alone or in combination with each of several post-emergent soybean herbicides (glyphosate, imazethapyr, imazamox, or fomesafen) of from 7% to 41%, with the dicamba/fomesafen combination lowering soybean yield more than any of the other combinations. This study is important in establishing yield losses from soybean exposure to realistic volatilization drift rates (e.g. 1%) under field conditions where such exposure is accompanied by application of common post-emergent soybean herbicides.

Tomatoes are even more sensitive to dicamba than soybeans. Recent experiments have established that a dose of dicamba of just 1.5 g/ha caused 5% flower loss at the early bloom stage of development, while 2.4 g/ha caused 5% flower loss at the early vegetative stage (Kruger et al. 2012). These rates are extremely low, representing roughly 1/300th to 1/200th of a typical application rate, respectively, and are well within levels that would result from drift and volatilization under field conditions. Only slightly higher levels were found to trigger a 10% loss in marketable fruit.

IV. Potential impacts of the requested registration on human health

A. Cancer

Epidemiological studies have associated dicamba exposure with increased incidence of a number of cancers in pesticide applicators. In 1992, epidemiologists with the National Cancer Institute (NCI) found that Iowa and Minnesota farmers who were first exposed to dicamba prior to 1965 had increased incidence of non-Hodgkin's lymphoma (NHL) relative to controls, with an odds ratio of 2.8 (Cantor et al. 1992, Table 6). A subsequent study in

Canada also established a correlation between exposure to dicamba and NHL (McDuffie et al. 2001). A study of cancer in Iowa farmers associated exposure to benzoic herbicides[35] with increased risk of multiple myeloma (Burmeister 1990).

Exposure to pesticides has long been suspected as a risk factor in NHL and multiple myeloma due to a striking fact. While farmers are generally healthier, and have lower overall cancer rates than the general population, they have higher than average risk of contracting NHL, multiple myeloma and several other cancers (Blair & Zahm 1995). This fact lends weight to epidemiology studies that find correlations between these cancers and and specific pesticides, such as dicamba.

Additional evidence comes from the Agricultural Health Study (AHS), a collaborative research project sponsored by the U.S. National Cancer Institute, the U.S. National Institute of Environmental Health Sciences, and the U.S. Environmental Protection Agency. The AHS follows the health status of a total of 52,395 pesticide applicators in Iowa and North Carolina. A 2006 study based on AHS data found suggestive associations between dicamba exposure and both lung and colon cancer (Samanic et al. 2006), with statistically significant exposure-response trends in both cases (Weichenthal et al. 2010).

B. Developmental Toxicity

There is also evidence, both epidemiological and experimental, suggesting that dicamba has developmental toxicity. An analysis of data collected in the Ontario Farm Family Health Study was conducted to assess the risk of birth defects in the children of parents exposed to pesticides in the three months prior to conception and the first trimester of pregnancy. The study found that pre-conception exposure to dicamba and cyanazine were associated with increased risk of birth defects in male offspring, with odds ratios of 4.99 and 2.42, respectively (Weselak et al. 2008). Pregnant mice that ingested drinking water spiked with very low doses of a commercial herbicide product containing dicamba, 2,4-D and mecoprop had reduced litter size, suggesting that this herbicide mixture has developmental toxicity (Cavieres et al. 2002). Greenlee et al. (2004) exposed preimplantation mouse embryos to low, environmentally relevant doses of a variety of agrochemicals *in vitro* for 96 hours. Exposure to 11 of the 13 agrochemicals, including dicamba (at 0.03 ug/ml), significantly increased the percentage of apoptosis in murine embryos. According to the authors, these results may have implications for human reproductive health. Higher rates of cellular death (apoptosis) in embryos could result in embryonic demise, implantation failures or alterations in the physiological processes underlying maternal recognition of pregnancy. Greenlee and colleagues offer their *in vitro* results as a possible explanation for the *in vivo* findings of Cavieres et al. (2002) noted above.

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[35] Dicamba is the most widely used benzoic acid herbicide.

C. Other health impacts

Researchers have also found a 20% inhibition of the nervous system enzyme acetylcholinesterase in a group of certified pesticide applicators whose only common pesticide used was dicamba (Potter et al. 1993). Acetylcholinesterase is an enzyme critical to neurological function. Children exposed to residues of organophosphate insecticides (which kill insects by disrupting acetylcholinesterase function) in foods have higher rates of attention deficit hyperactivity disorder (Bourchard et al. 2010), a condition afflicting 4.5 million children in the U.S. (Monday 2010). Exposure to dicamba may have similar effects.

A study of the frequency of sister chromatid exchanges (SCEs) and cell-cycle progression assays revealed that high doses of dicamba can damage DNA, leading the study authors to warn that dicamba is a "potentially hazardous compound to humans" (Gonzalez et al. 2006), though the relevance of the high doses tested to actual human exposure is difficult to assess. Another study that examined dicamba after its activation by plant (dicamba–treated corn) or animal (liver microsomes) found it to be mutagenic in standard bacteria and yeast assays (Plewa et al. 1984). A fuller description of the potential health impacts of dicamba may be found in Cox (1994).

D. Increasing exposure through multiple routes

EPA has set the dietary chronic reference dose[36] for dicamba at 0.45 mg/kg/day, based on a reproductive study on rats that found a lowest observed adverse effect level (LOAEL) of 136 mg/kg/day, a no observed adverse effect level (NOAEL) of 45 mg/kg/day, and 10X safety factors for both interspecies extrapolation and intraspecies variation (US EPA 2006, p. 7, see study MRID 43137101 in Appendix D). This study was conducted by Huntingdon Reseach Centre, the U.K subsidiary of Huntingdon Life Sciences, a contract research organization that performs extensive safety testing of pesticides on contract with major pesticide firms.

EPA also estimated dietary exposure to dicamba from water and food. Chronic dietary exposure was estimated at 0.0118, 0.0199 and 0.0297 mg/kg/day for the general population, infants and children 1-2 years of age, respectively (US EPA 2006, p. 9).

In 1987, EPA issued a Health Advisory for dicamba that was based on studies not cited in the 2006 reregistration eligibility decision (US EPA 1987). Based on a two-year study (Davis et al. 1962) in which beagle dogs were fed dicamba at various doses (0, 0.125, 0.625 or 1.25 mg/kg/day) for two years, which found reduced weight in males at 0.625 and 1.25 mg/kg/day, and in females at 1.25 mg/kg/day, EPA identified an NOAEL of 0.125

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[36] This is the presumed level of a pesticide that can be consumed daily for up to a lifetime without an appreciable risk of deleterious effects. The official EPA definition is: "An estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure for a chronic duration (up to a lifetime) to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime."

mg/kg/day. Application of an uncertainty factor of 100X yielded a chronic (lifetime) reference dose of 0.0013 mg/kg/day.

This value is over two orders of magnitude lower than the 0.45 mg/kg/day chronic reference dose utilized in the 2006 reregistration eligibility decision. As noted above, the EPA estimates dietary exposure to dicamba at 0.0199 mg/kg/day for infants and 0.0297 mg/kg/day for young children. Both values exceed the chronic reference dose of 0.0013 mg/kg/day identified by EPA in the 1987 Health Advisory. The 1987 EPA document also cites a 1977 National Academy of Sciences report in which the NAS committee set the acceptable daily intake (ADI)[37] for dicamba at 0.00125 mg/kg/day, very close to the reference dose established by EPA in 1987. EPA should re-adopt this more protective standard to best protect infants and children.

The discussion above suggests that exposure to dicamba is likely already having adverse health impacts on farmers, infants and children. Granting the requested registration would lead to dramatically increased exposure to this herbicide and exacerbate these health impacts.

EPA's assessment of occupational risk assumes just one application of dicamba per growing season (EPA 2006, pp. 5, 13), while the maximum single application and in-crop seasonal limit is just 0.25 lb/acre for cotton (BASF 2010). Dicamba would be used more frequently, later in the season, and at higher doses with MON 87701 than with any prior use of the herbicide on any crop. This would sharply increase farmer exposure during handling and application (dermal, oral and inhalational). Novel post-emergence through pre-harvest use of dicamba in cotton would sharply increase post-application exposure when workers re-enter the field after application. Vaporization of volatile dicamba from plant surfaces, especially given the large surface area of plants sprayed post-emergence to pre-harvest, would increase both inhalational and dermal exposure. EPA must take full account of these factors as it considers the requested registration.

EPA notes that dicamba tolerances in 1985 ranged from 0.05 to 40 ppm on a variety of agricultural products (US EPA 1987). Dietary exposure to dicamba in food has likely increased substantially since that time, given the expanded range of agricultural products with dicamba tolerances, and their generally higher levels. CFS counts dicamba tolerance values of equal to or greater than 40 ppm in fully 11 agricultural products, including the forage and hay of forage grasses, group 17 (at 125 and 200 ppm, respectively), and of wheat, oat, rye, teff and millet forage (90 ppm), in addition to aspirated grain fractions (1000 ppm), which are often fed to animals. The rising feed animal exposure to dicamba implied by these rising tolerances translates into greater dicamba residues in the organs, meat byproducts, and meat from such animals.

Human exposure to dicamba residues in these products would increase still further if the requested tolerance for cotton, gin byproducts of 70 ppm is granted. A 2001 technical report by researchers at Texas A&M states that fully 30-48% of cotton gin byproducts

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[37] Use of the term ADI has been superseded by reference dose, but the meaning is essentially the same.

(CBG) are fed to cattle, with 15-23% of cattle feedlots accounting for this use of CBG. Huge amounts of CBG are produced as a byproduct of cotton production, roughly 150-200 lbs. per 480 lb. bale. According to one estimate, 500,000 to 700,000 tons of CBG (also called gin trash) are produced in states east of the Mississippi River each year, which could potentially feed 300,000 to 400,000 cows for 100 days with little additional supplement (Stewart 2010). CFS notes the potential for a further increase in exposure if Monsanto's pending requests for new dicamba tolerances of 45 and 70 ppm for soybean forage and hay, respectively, are granted.

Water may be a still more important source of dietary exposure. Dicamba is moderately persistent in soil and water, and is frequently found contaminating ground and surface waters, for instance in 28% of rivers sampled in the U.S. (Thurman et al. 2003). In 1987, EPA reported detection of dicamba in a significant proportion of surface water samples (249 of 624) and ground water samples (39 of 275) at levels up to 3.3 ug/L and 0.8 ug/L, respectively (US EPA 1987). Dicamba will become much more prevalent in surface and ground water, and at much higher levels, if the requested registration is granted, leading to increased exposure in drinking water.

Given the likelihood of adverse human health impacts from current levels of exposure to dicamba, as discussed above, and the many-fold increase in dicamba use and exposure to be with MON 87701, EPA should reject the requested registration.

V. Environmental impacts of dicamba use with MON 87701 cotton

Overview of environmental impacts

Herbicide resistant crops are not the ultimate cause of increased conservation tillage, nor are they required to maintain conservation tillage. EPA should not succumb to the arguments that use purported benefits of conservation tillage to counterbalance harms of increased dicamba use. First we analyze the relationship of conservation tillage to herbicide use with HR crops, and then environmental impacts of dicamba use on MON 87701.

A. Herbicide resistant crops not responsible for increased conservation tillage

Contrary to prevalent misconceptions, herbicide-resistant crop systems have not driven any meaningful increase in the use of conservation tillage. The following discussion may be summarized as follows.

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

Second, to the extent there is such a correlation, the causation is from prior adoption of conservation 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 use 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 often attributed to conservation tillage are disputed in the scientific community, while in other cases this form of tillage appears to have adverse impacts.

1) Correlation in question

One often sees reference to 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).

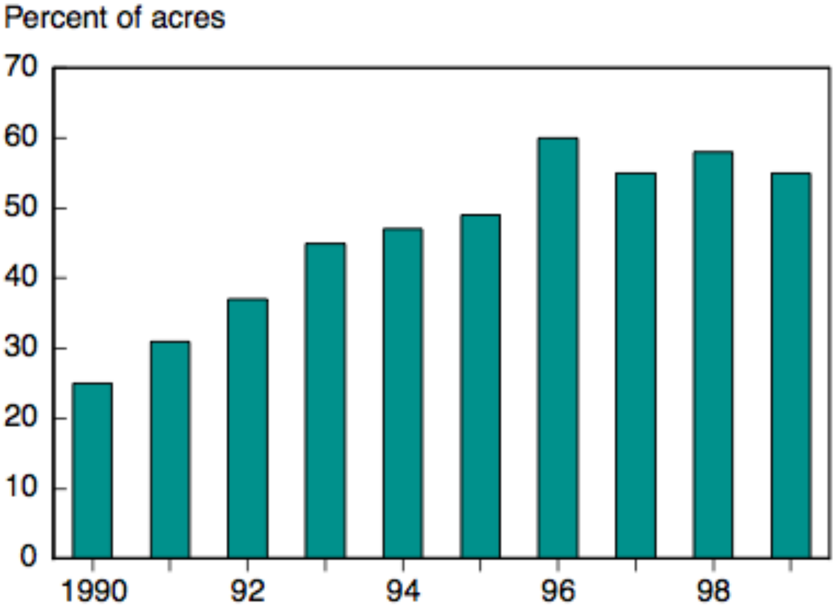
Despite this caution, the authors proceed to base their discussion of purported reductions in soil loss and compaction from GR crop systems almost entirely on a survey conducted by the American Soybean Association (ASA) in 2001, a survey that was not subject to peer review or published in any "refereed publication." These ASA survey results are extremely difficult to reconcile with an assessment conducted the following year by USDA agricultural economists Jorge Fernandez-Cornejo 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 least 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.

2) HR crop adoption does not drive increased use of conservation tillage

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. 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 below.

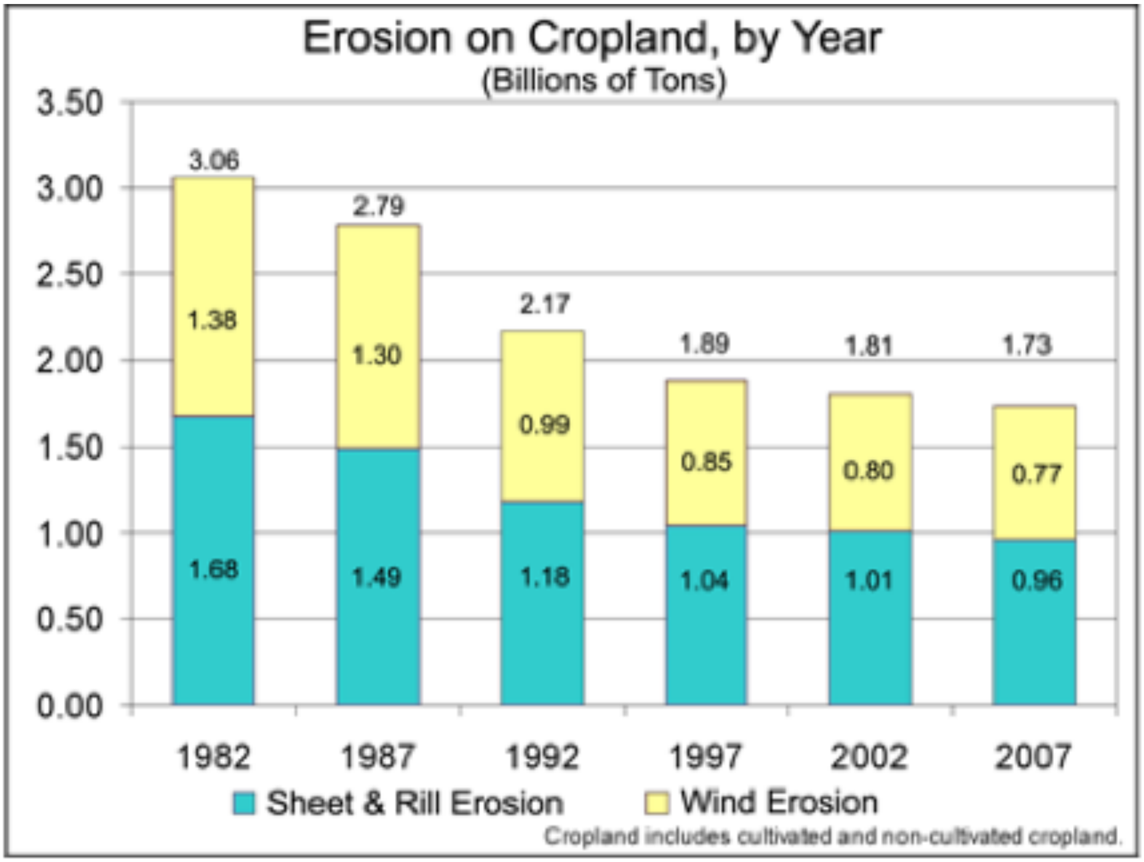
3) Reductions in soil erosion come to an end during the decade of herbicide-resistant crop adoption

Conservation tillage is widely credited with bringing about large reductions in soil erosion rates. Thus, if HR soybeans, corn and cotton did in fact promote greater use of conservation tillage, one would clearly expect to see sharply falling soil erosion rates over the period of their widespread 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 this 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 based upon long-term climate data, inherent soil and site characteristics, and cropping and management practices."

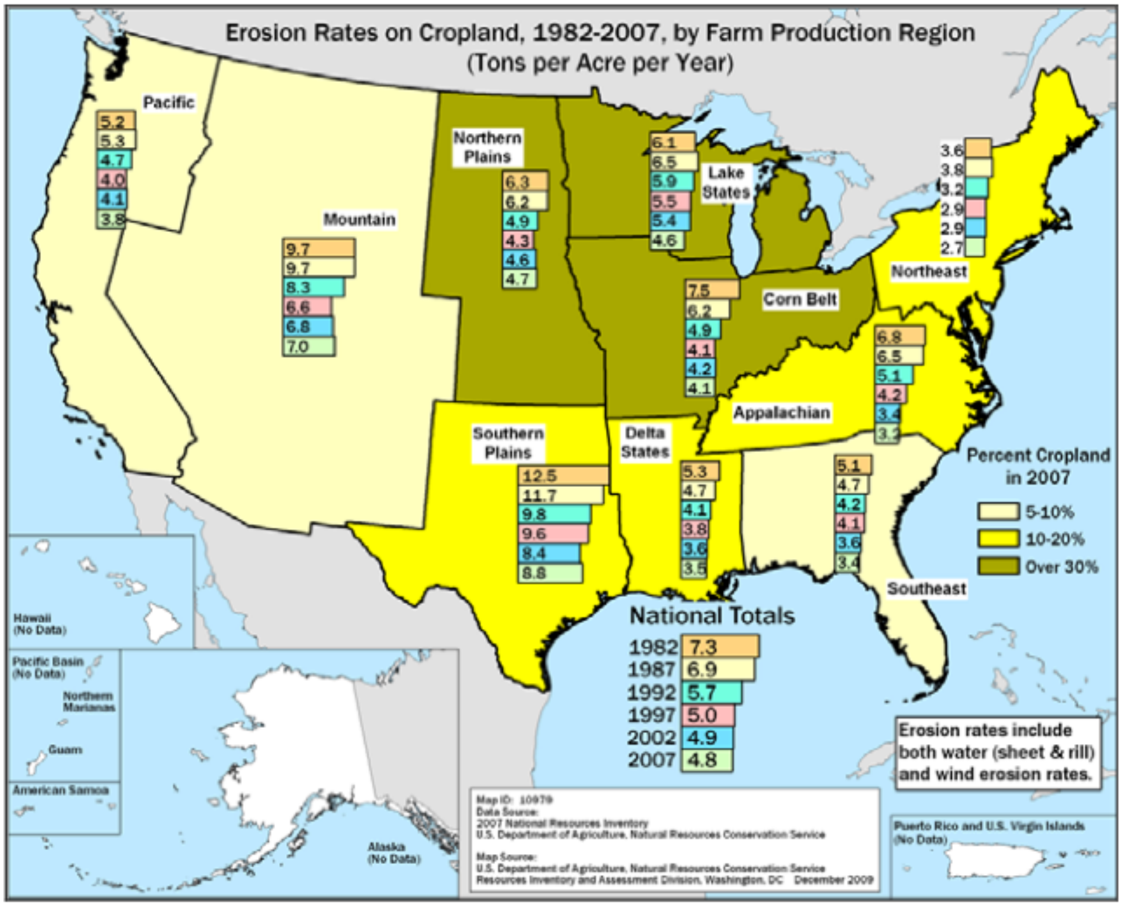
Tillage regimes are the primary component of "cropping and management practices," and thus play a large role in determining soil erosion rates. It is well established that soil erosion increases with the intensity of tillage, and decreases as farmers adopt regimes that leave more plant residue on the soil (USDA ERS AREI 2002). Thus, the chart below reflects in large degree the tillage regimes used by farmers.



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 steadily from 16.0 million acres in 1997 to 117.2 million acres in 2007 (Benbrook Supplemental 2009a, Table 5). If HR crops promoted adoption of conservation tillage in any significant way, one would surely expect a much stronger decline in soil erosion over a period when their adoption increased by 100 million acres.

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



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

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

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

The Corn Belt states (Iowa, Illinois, Indiana, Missouri and Ohio) and the Northern Plains states (Kansas, Nebraska, North and South Dakota) comprise two-thirds of the nation's corn and soybean acreage, and all of these states have high adoption rates of GE herbicide-resistant soybeans and corn. If the supposition that HR crops and HR soybeans 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 than the national average. However, this is not the case at all.

In the Corn Belt states, the annual erosion rate remained constant at 4.1 tons per acre from 1997 to 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 than stable or increasing erosion rates.

4. 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 in ALL farm production regions, including Corn Belt and Northern Plains states, in the 1982 to 1997 period before any appreciable adoption of HR crops? The 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 increased use of conservation tillage. According to Coughenour and Chamala (2000), authors of a book examining the history of conservation tillage in the U.S. and Australia:

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

5. 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.*** (Cox et al. 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 on erodible land. 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:

> 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 2006, p. 3).

"Residue management practices" refer to conservation tillage practices.

These results indicate that more frequent and extensive use of dicamba with MON 87701 cotton will have an impact on levels of dicamba found in diverse water bodies in watersheds where soybeans are grown, and that these new impacts need to be assessed for their potential to injure non-target species of both plants and animals.

B. Environmental impacts of conservation tillage

Even if using dicamba with MON 88701 cotton is part of a conservation tillage program for some growers, the environmental benefits attributed to reduced tillage are generally not well substantiated.

1) 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 (Malhi 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 use of dicamba with MON 88701 cotton were to increase use of conservation tillage in the short term.

2) Climate change

It is often claimed that continued use of conservation tillage associated with HR crops will reduce greenhouse gas (GHG) emissions as a result of increased carbon sequestration in soils, decreased fuel consumption, and the reduction of nitrogen soil amendments. These benefits for climate change of a purported preservation of no-till 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).

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, relying on conservation tillage as an argument for a whole range of environmental benefits of HR crops and herbicides without critical analysis of the best science available is unfounded.

In fact, overall environmental benefits and harms from use of dicamba with MON 88701 cotton are likely to have more to do with changes in herbicide use that accompany this cropping system than with tillage methods that may or may not be different.

C. Environmental effects of increased dicamba use with MON 88701 cotton

1) Injury to plants and other non-target organisms from exposure to dicamba

Dicamba is a particularly potent poison for many species of plants, especially dicotyledons (dicots, or broadleaf plants) that are sensitive to very low drift levels and to dicamba-contaminated water in semi-aquatic areas (US EPA 2006) p. 18 - 19, as also discussed in our comments on injury to crop plants above. Dicot species vary in their sensitivity to dicamba, e.g. (Johnson et al. 2012). Even monocots such as members of the grass and lily families can be killed by off-site movement from labeled doses of dicamba, and suffer sub-lethal injuries from drift levels at certain times in their life cycles (US EPA 2005, 2006).

a. Injury from dicamba in water

In reregistering dicamba, EPA said: “Based on fate characteristics, dicamba and DCSA would be somewhat persistent in aerobic and anaerobic conditions and would be expected to be persistent in groundwater” (US EPA 2006) p. 14. Dicamba has in fact been detected in ground water, although the quality of sampling is considered unreliable because measurements were not necessarily taken in areas where dicamba was in use, and detection levels were often unknown and probably high (US EPA 2005) p. 39, which would tend to underestimate the extent of contamination.

Dicamba is more frequently detected in surface waters, however. Water sampling in rivers by USGS bears out the propensity of dicamba to enter and persist in water bodies, even in numbers of samples that are disproportionately high compared to the extent of dicamba use (Thurman et al. 2001). In this 2001 study, USGS researchers developed a more sensitive and less laborious method to detect dicamba and 2,4-D in water, saying that previous methods made it difficult to conduct reliable extensive sampling. They applied their better method to river water samples from 14 areas across the US taken as part of the US Geological Survey’s National Water Quality Assessment (NAWQA) program, and selected to represent different leaching and runoff scenarios, after rains during the peak herbicide application season. Surprisingly, although 5 times more 2,4-D than dicamba was used agriculturally, dicamba was detected in more samples – 28% of samples for dicamba vs. 16% of samples for 2,4-D. The highest percentage dicamba samples came from a corn and soybean region of the White River of Indiana, whereas the 2,4-D samples were most common in the wheat-growing region of the Red River.

Plants can take up dicamba via water through their roots or from exposure of above-ground plant parts to the contaminated water, putting sensitive terrestrial plants in riparian areas and semi-aquatic wetland areas at particular risk from increased use of dicamba with MON 88701 cotton (US EPA 2005).

Aquatic animals may also be at increased risk after applications of dicamba formulations to MON 88701 cotton. In reregistering dicamba, EPA examined databases where ecological incidents were reported, and there were some aquatic cases:

> Approximately, 100 incidents have been reported associated with dicamba usage. Incidents reported include impacts to terrestrial and aquatic non-target plants and animals. The majority of reported incidents are damage to plants including a wide range of crops (corn, sorghum, soybeans, sugar beets and wheat) as well as impacts to non-crop plants. The specific impacts varied from browning and plant damage to mortality of all plants within the treated area. Aquatic impacts reported consist of three fish kill incidents associated with pasture and residential turf application. (US EPA 2005, p. 6)

Looking at the more complete description of incidents, one fish-kill occurred after an agricultural application. Fish kills were also reported from turf and pasture applications.

The reasons for the fish-kills are not discussed. Dicamba is reported to have only slight acute toxicity to fish (US EPA 2006) p. 16, so perhaps surfactants were the toxic agent (Cox 1994). Chronic and sub-lethal toxicity of dicamba to aquatic organisms have not been studied (US EPA 2006, p. 16).

These results indicate that more frequent and extensive use of dicamba with MON 88701 cotton will have an impact on levels of dicamba found in diverse water bodies in watersheds where cotton is grown, and that these new impacts need to be assessed for their potential to injure non-target species of both plants and animals.

b. Injury to plants from spray drift and volatilization of dicamba

Reports of injury to non-target crops from dicamba spray drift and volatilization are common, even from registered existing uses of dicamba, as discussed in our comments on this topic, above.

If dicamba is moving off-site far enough to cause injury to crops, it is undoubtedly also causing injury to wild plants. Drift of dicamba 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 dicamba, injury has also occurred at locations distant from the application site, putting organisms in a variety of natural areas at risk.

Note that EPA only models spray drift when determining risks, because volatilization is too difficult to model (US EPA 2005), although it is an acknowledged hazard. In fact, the European Food Safety Authority has this as a "Critical area of concern" for dicamba use: "[d]icamba has the potential for long-range transport through the atmosphere." (EFSA 2011, p. 14)

It is likely that even crop injury from pesticide moving off-site 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. It is clear that non-target organisms do risk injury from dicamba used in agriculture, and that use dicamba with MON 88701 cotton is likely to increase that risk to the extent that the crop system involves increased use and changes in patterns of use of dicamba.

c. Injury from the effects of off-site pesticide movement on pests and pathogens

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).

EPA thus needs to assess the impact of increased use of dicamba with MON 88701 cotton on the pests and pathogens of non-target plants.

d. Injury to terrestrial wild animals from off-site movement of dicamba

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

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

> application rate, and nine scenarios at the highest application rate. (Bautista 2005, p. 22)

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

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

These concerns about dicamba impacts on wild animals will only be amplified by the increased use of dicamba with MON 87708 cotton.

2) Injury to animals from ingestion of dicamba residues and metabolites from use of dicamba with MON 87708

a. New types of metabolites and different ratios of residues and metabolites are present dicamba-treated MON 87708

Dicamba applications with MON 88701 cotton will also leave dicamba residues and metabolites in the MON 88701 cotton tissues, including some metabolites that may be unique compared to those in non-MON 88701 cotton. Also, there may be more wild food plants that take dicamba and metabolites from contaminated water and soil at higher levels with use of dicamba on MON 88701 cotton.

In order to determine how applications of dicamba to MON 88701 cotton will affect the kinds and amounts of residues and metabolites, it is essential to have data on the kinds and amounts of dicamba residue and metabolites in non-dicamba resistant cotton treated with dicamba for comparison. CFS was unable to find this information in primary published reports. However, detailed data from industry studies on dicamba residues and metabolites in non-dicamba-resistant cotton and other plants (Butz and Atallah 1982, as cited in Yamada 2010) is available from the FAO (Yamada 2010), along with summaries of these data (FAO 2011).

Plants – both those that are sensitive to dicamba, such as cotton, and those with natural tolerance, such as corn and wheat – take up dicamba from leaves, stems and roots, and then translocate it to growing regions, such as seeds and other reproductive parts. But most of the dicamba that enters the plant remains as the parent herbicide. Metabolism results in a variety of compounds:

> Despite some differences in the rate of metabolism and translocation, there seems to be a common metabolic pathway of dicamba after its foliar application to these four plant species. The metabolism of dicamba appear to follow: hydroxylation of dicamba at the 5-position to form 5-OH dicamba; O-demethylation of 5-OH dicamba to form DCGA; O-demethylation of dicamba to form DCSA; O-demethylation of dicamba and hydroxylation to form DCGA; and conjugation of 5-OH dicamba and DCSA with glucose to form the ß-D-glucosides. (FAO 2011, p. 181)

Below is a diagram of the proposed pathway of dicamba metabolism in plants (Yamada 2010, p. 970):

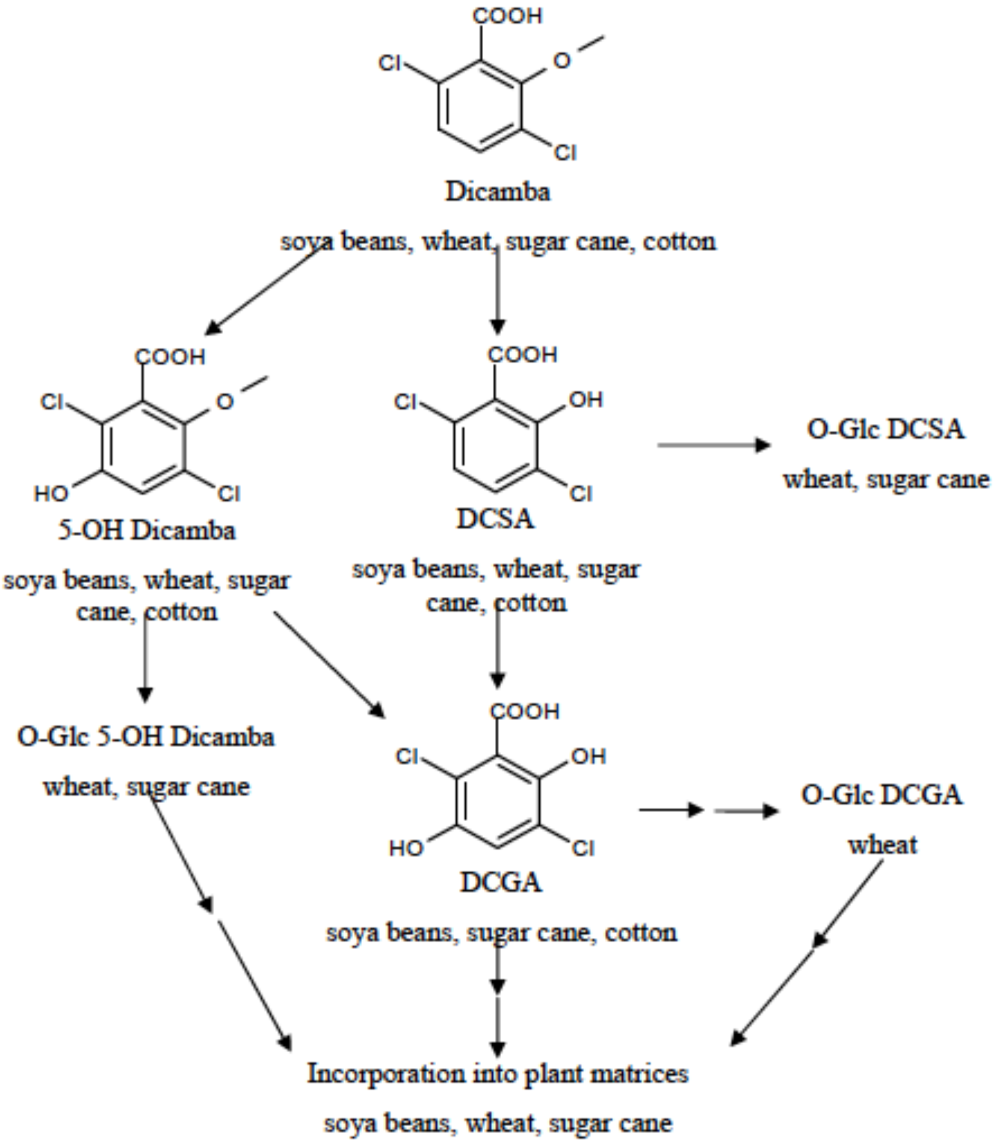


Figure 2 Proposed metabolic pathway of dicamba in plants

For studies of dicamba metabolism in cotton, radiolabeled dicamba was applied to leaves at the "green boll stage" at a low rate that allowed plant development to continue (Butz et al. 1982, 1984; as cited in Yamada 2010, p. 964). Residues and metabolites were measured at different times after the applications, and in different plant tissues. Over time, dicamba was translocated throughout the cotton plant: "….in spite of volatilisation [from the treated leaves], a significant portion of applied radioactivity translocated to the untreated leaves, stems, roots, and especially to the immature bolls…Approximately one-half of the radioactivity was found in the immature bolls…" where it was found in lint, seeds, and at highest levels in carpels (Yamada 2010, p. 965).

Most of the recovered radioactivity in all tissues was in parent dicamba, with small amounts in DCSA found in some parts of the plant. In leaves, very low levels of the metabolite DCSA were measured at the earlier sampling times, and both DCSA and DCGA were measured at very low levels at later times after application. Some 5-OH dicamba was also detected. Conjugates of DCSA and DCGA were not reported. The data presented are presented in full (Yamada 2010) p. 964 – 967, and are summarized in the final FAO report:

> Dicamba was the predominant radioactive residue in ether fractions of treated seed (14 DAT [Days After Treatment]) at 2.2% of TAR [Total Applied Radioactivity]. Further analysis indicated that dicamba was poorly metabolized or not conjugated in cotton seed.
>
> Dicamba was the predominant residue in all analysed cotton parts with very slow metabolization showing minor amounts of 5-OH dicamba. (FAO 2011, p. 180)

These data on kinds and relative amounts of residues and metabolites in non-dicamba-resistant cotton should be the baseline for comparison with MON 88701 cotton.

CFS does not have access to Monsanto's Nature of Residue and Magnitude of Residue studies for MON 88701 cotton. However, we expect the metabolism of dicamba in cotton to be similar to dicamba metabolism in soybean, and we do have some information about soybean, both conventional and dicamba resistant.

Conventional soybean has a similar pattern of dicamba metabolism as cotton. As in cotton, for studies of dicamba metabolism in soybean, radiolabeled dicamba was applied to leaves of soybeans at different stages of development and at a low rate that allowed plant development to continue. Residues and metabolites were measured at different times after the applications, and in different plant tissues. In summary, most of the recovered radioactivity was in parent dicamba, with small amounts in DCSA. Low levels of the metabolite DCSA were measured at the earlier application timings, and both DCSA and DCGA were measured at very low levels at later applications. Conjugates of DCSA and DCGA were not detected. The data are presented in full (Yamada 2010) p. 954 – 957, and are summarized in the final FAO report:

> [14C]-Dicamba was applied at a sub-toxic rate of 5.17 µg/plant to soya bean plants (foliar application) grown on untreated soil at two different timings.
>
> With the early podfill growth stage application, radioactivity rapidly decreased from 85% to 4.6% of the total applied radioactivity (TAR) in treated leaves in the first seven days after application. After 14 days, the total recovered radioactivity averaged 42% of the TAR, about a half of which was found in immature beans. This indicates rapid and significant incorporation and translocation from leaf to beans.
>
> Over 64% and 94% of respective TRR [Total Recovered Radioactivity] were attributed to unchanged dicamba in treated leaves and immature beans 14 days after treatment. About 17% of the TRR in the leaf samples collected 14 days after treatment (DAT) was attributed to DCSA while **only 0.6% of the TRR in the 14-DAT immature bean samples was DCSA.** No di-hydroxylated metabolites were observed.
>
> The result indicates that dicamba is translocated without metabolization or conjugation while at the site of application, dicamba goes through gradual O-demethylation.
>
> With late senescent growth stage application, also rapid decline of radioactivity from 77% to 11% of TRR was observed in treated leaves 6 days after application. Only 63% of the TAR was recovered in the plant 6 days after treatment, among which 26% was found in the intact plant while another 24% was recovered from abscised leaves.
>
> Untreated leaves, stems, roots, pods and immature beans 6 days after treatment contained similar radioactivity. Their radioactivity levels were similar also to those of the same tissues after early podfill stage application except immature beans. With late senescent growth stage application, there was far less translocation of dicamba to beans compared to early podfill stage application. Only 2.1% of TAR or 8% of the TRR remained in immature beans 6 days after treatment. (FAO 2011, pp. 179 - 180)
>
> About 64% and 44% of respective TRR were attributed to unchanged dicamba in treated leaves (not abscised) and beans 6 days after treatment. **Only 0.3% and 0.7% of the respective TRR were attributed to DCSA** in 6-DAT bean sample and treated leaves (not abscised**). Similarly small amounts of 5-OH dicamba and DCGA were also found in treated leaves and immature beans but neither exceeded 1.0% of TRR** (p. 179).

Engineered dicamba-resistant plants have a transgenic enzyme that metabolizes dicamba, so are expected to have a different quantitative and/or qualitative profile of metabolites. For dicamba-resistant soybean, MON 87708, CFS was unable to obtain Monsanto's Nature of Residue study, but a summary of the findings was included in their Magnitude of Residue study (Moran and Foster 2010):

> The study of the nature of the residue (Miller and Mierkowski, 2010) demonstrated that, in dicamba-tolerant soybean**, dicamba is metabolized mainly to a glucose conjugate of 3,6-dichloro-2-hydroxybenzoic acid (DCSA), with smaller amounts of conjugates of 2,5- dichloro-3,6-dihydroxybenzoic acid (DCGA) and another glucose conjugate of DCSA.** Dicamba was present at very low levels. Another metabolite, 2,5-dichloro-3-hydroxy-6-methoxybenzoic acid (5-hydroxydicamba), which is observed in corn, was not found as a metabolite of dicamba in dicamba-tolerant soybean. The conjugates are very complex molecules which are not readily synthesized to produce analytical reference standards. (Moran and Foster 2010, p, 30)

From this summary, it appears that the major metabolites of dicamba present in MON 87708 soybean are not found in the metabolism studies of non-dicamba-resistant soybean, namely glucose conjugates of DCSA and DCGA. These conjugates are present in other crop plants that were studied, but at very low levels, rather than being the major products, as here. And, although the toxicity of DCSA and DCGA has been studied, apparently the toxicity of the conjugates has not been studied.

Note that in the Magnitude of Residue studies, the DCSA and DCGA conjugates are converted to DCSA and DCGA for measurements, so the levels of the unique and untested conjugates are not discernable in the results (Moran and Foster 2010).

Besides these qualitative differences, the relative levels of parent dicamba to its metabolites is “flipped” in MON 87708, with dicamba being low and metabolites high, instead of the other way around.

Of course, given how little dicamba is used in soybeans today, and then only pre-plant or just before harvest, little dicamba or metabolites are ever detected (Yamada 2010).

Assuming that MON 88701 cotton behaves similarly to MON 87708 soybean, we expect that glycosides of DCSA will be the main metabolites of dicamba that result from activity of the engineered enzyme in MON 88701 cotton. Studies of conjugated metabolites of 2,4-D, such as dicholorphenol (DCP) glycosides, show that the DCP aglycone can be released during mammalian digestion with possible impacts on health (Laurent et al. 2000, 2006; Pascal-Lorber et al. 2003, 2008, 2012). Free DCSA may also be released from conjugates during digestion. These conjugates in MON 88701 cotton thus need to be measured and tested for toxicity, and included in the dicamba tolerances for food and feed, along with free DCSA.

Another concern is whether the formaldehyde produced in the breakdown of dicamba by the engineered DMO enzyme in MON 88701 cotton when dicamba is applied results in formaldehyde levels over and above those that naturally occur in cotton, and that may be injurious to animals that eat the plant parts, since there can be health effects from ingestion of formaldehyde (ATSDR 2008, Fig. 1.2). Formaldehyde levels in dicamba treated MON 88701 cotton tissues should be tested after applications to see if they fall below or above safe limits.

EPA must assess the impacts of these substantial differences in kinds and amounts of dicamba residues and metabolites when dicamba is used with MON 88701 cotton compared to control cotton plants, for both human health and for wildlife.

b. Wild animals, including pollinators and other beneficial organisms, are at particular risk from dicamba residues and metabolites

Food safety assessments based on residue and metabolite studies for humans are not appropriate for wildlife. Humans eat the processed products from seeds of cotton, and tissues from animals that have eaten cotton seed parts or cotton forage, whereas wild animals may eat any part of the plant, including pollen and nectar (pollinators and other beneficial animals). Feed for livestock, such as hay and forage, is likely to have higher herbicide residues than do seeds, but it is assessed for feed safety after the approved waiting time post-application, whereas wild animals may eat the these vegetative tissues immediately after applications and thus receive a much higher dose. Residues and metabolites in pollen and nectar used by pollinators and other organisms beneficial to agriculture are not taken into account in food and feed assessments, either.

It is safe to assume that the engineered DMO enzyme is active in reproductive parts including pollen because MON 88701 cotton apparently can be sprayed with dicamba after flowers have formed without significant injury (Brinker et al. 2012). It is thus likely that metabolites will be present in pollen and nectar as a result of dicamba translocation into floral parts from elsewhere in the plant. In cotton there are also nectaries located outside of the flowers (extrafloral nectaries), as discussed below, that because of their high metabolic activity are likely to receive translocated dicamba. Residues and metabolites of dicamba should therefore be measured in floral parts and extrafloral nectaries.

Cotton plants attract pollinators and other nectar- and pollen-eating animals, including honeybees and wild bees of several genera (Borem et al. 2003), beneficial wasps that parasitize herbivorous insects (Röse et al. 2006), and both beneficial and pest species of moths and butterflies (Röse et al. 2006). Not only are these insects attracted by the large flowers with both pollen and nectar, but also cotton plants have nectaries outside of the flowers that produce nectar both before and after flowers open, extending the season that cotton plants are attractive.

Potential harmful effects of dicamba and its metabolites in MON 88701 cotton on honeybees and the honey produced from their collection of pollen and nectar are of particular concern. For example, a beekeeper describes how honeybees move to different nectaries:

> Honey bees forage cotton on both its internal and external nectaries. Honey bees collect nectar secreted inside the pale yellow flowers on the first day of their bloom. After one day, the flowers change colors, becoming dark pink. The honey bees learn that the change of flower colors means they must move to the external nectaries. The honey bees then collect nectar from the green-colored bracts, leaf-like flower parts on the base of the flower [calyx and epicalyx]. They also find nectar secreted from the under-side of the leaves. (Underhill 2010)

There are actually five sets of nectaries on cotton plants, one set at the base inside the flower, and then:

> …a) three triangular nectaries surrounding the calyx, next to the base; b) three nectaries in the pedicel of each flower, below each bract of the epicalyx; c) a nectary on the basal part of each flower; d) unipapilled nectaries on the floral peduncle and stem of young leaves. While the floral nectary secretes nectar only on the day the flower opens, the extra-floral nectaries do this for a few days. Although it has been observed that bees prefer to visit the floral nectaries, the extra-floral ones are important to maintain the bees in the culture before the flowers open (Moffett it al., 1979) and to attract part of the harmful insects, keeping them away from the flowers (McGregor, 1976). (Borem et al. 2003)

If dicamba is used on MON 88701 cotton during flower development, or if dicamba or metabolites are redistributed in the plant - from earlier applications - during growth, presence of dicamba and metabolites 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 and other herbicides also travel 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 MON 88701 cotton may be protected from the toxic effects of dicamba because of the expression of DMO, and so may accumulate more dicamba and metabolites than non- MON 88701 cotton flowers would: 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).

Surprisingly, the DMO enzyme itself may retain its activity in honey, even during digestion by immature bees, able to degrade herbicides brought in from sources other than MON 88701 cotton (for example, from dicamba-resistant soybean and corn) into metabolites (Grogan and Hunt 1979).

One of the only studies to examine changes in nectar after introducing a defense compound via genetically engineering is relevant here (Sala Junior et al. 2008). Monsanto asserts that formaldehyde (a metabolite of dicamba in MON 88701 cotton) and salicylic acid (similar to DSCA, also a metabolite of dicamba in MON 87708) may trigger plant defense in soybean (Monsanto 2010, p. 250), and thus presumably in cotton. EPA thus needs to consider the toxicity to beneficial organisms of defense compounds made in nectar of MON 88701 flowers in response to dicamba applications, as Sala Junior and colleagues did in citrus.

Sweet orange trees were genetically engineered to express an antibacterial peptide, sarcotoxin IA, to see if this peptide protected the orange trees against bacterial citrus canker. Because orange blossoms attract a variety of pollinators, and nectar composition is important to the health of pollinators, these researchers wanted to know if there were differences in nectar constituents:

> Nectar varies in chemical composition and these characteristics reflect the type of pollinator (Baker and Baker 1983). Also, the nectar is not sterile. Insects or avian pollinators certainly transfer microorganisms from flower to flower and, for this reason, a chemical variation in the nectar composition could alter the microflora present in the nectar… Results so far suggest that transgenic plant impacts on pollinators will depend on a case-by-case analysis of the gene concerned and its expression in the parts of the plant ingested by insects (Malone and Pham-Delegue 2001). Considering this aspect, studies of the nectar chemical composition are important to access environmental impacts of transgenic plants. (Sala Junior et al. 2008) p. 2.

And they did, in fact, find difference that could impact pollinators:

> In summary, the floral nectar components of the conventional and transformed STX IA sweet orange trees were analyzed to study possible quantitative and qualitative modifications. The results showed that there are significant differences in the primary and secondary metabolites contents. These data suggest that the introduction of the gene responsible for the production of the antibacterial peptide sarcotoxin IA could modify the amino acids, triacylglycerides and purine alkaloids contents present in the sweet orange nectar. Such nectar with altered composition may affect floral visitors, such as nectar robbers, generalist pollinators and specialized pollinators. This work shows that deeper investigations are required to enlarge our understanding of multispecies interactions, as plant–pollinator, plant–herbivore and plant–microorganisms and to evaluate the impact of gene insertions on the nectar composition of genetically modified plants. (Sala Junior et al. 2008 p. 6)

Finally, since cotton pollen is collected along with nectar, dicamba and its metabolites need to be assessed in pollen after dicamba treatment of MON 88701 cotton. Although transgene expression is sometimes reported during risk assessments for Bt crops because the Bt protein is toxic to many butterflies (Malone and Pham-Delègue 2001, Mattila et al. 2005, Peterson et al. 2006), levels of pesticides associated with herbicide-resistant crops have not been measured in pollen of these crops.

In summary, EPA needs to reconsider potential increased risks to wild animals of various types from eating MON 88701 cotton tissues after applications of dicamba, or drinking runoff, in light of herbicide use projections, taking into account the difference between human or livestock exposure vs. wild animal exposure.

In particular, to properly consider any unreasonable adverse effects of using dicamba with MON 88701 cotton, EPA must consider how such use may have an unreasonable adverse effect on pollinators, which are organisms beneficial to agriculture. Taken together, the fact that honeybees are likely to collect pollen and nectar of MON 88701 cotton after it has been sprayed with dicamba, that resulting residues and metabolites are likely to be different from those in conventional cotton since such cotton is not treated with dicamba during growth and does not metabolize dicamba in the same way, and that some of these metabolites may trigger defense responses that have impacts, we request that the EPA consider the impacts to honeybees and other pollinators. There are guidelines for analyzing impacts of transgene products on bees set out by Malone and Pham-Delègue, for example (2001, p. 299 – 300).

3) Impacts to non-target species of dicamba use patterns with MON 88701 cotton

When dicamba is used during the season is also an important factor in determining risk of injury to non-target organisms. Simply, the amount of injury that non-target organisms will sustain is determined by how sensitive they are to the dicamba formulation and by the dose they receive. Plants are more sensitive to dicamba at some stages of development than at others. Because MON 88701 cotton has been engineered to withstand dicamba, thus removing biological constraints, this herbicide can be used during the main growing season for the first time.

a. Timing of dicamba 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 MON 88701 cotton itself is less sensitive to injury during spring and summer than is non- MON 88701 cotton. This is a general outcome of herbicide-resistant crop systems: "The role of total postemergence programs to control grass and broadleaf weeds has expanded with the development of herbicide-resistant 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). This has been clearly shown with dicamba and injury to tomato plants (Kruger et al. 2012). Drift levels of dicamba have also been shown to affect asexual reproduction in potatoes (Olszyk et al. 2010), and seed production in peas (Olszyk et al. 2009), sometimes without accompanying vegetative injury.

b. Total use of dicamba at the landscape level

Another way that dicamba use will increase with MON 88701is by increasing the total number of cotton acres that are treated with this herbicide. Within a given year, many more cotton acres will be sprayed, as we show in our comments on herbicide use. Also, since cotton and corn or soybeans are sometimes rotated on the same acreage within a region, it is likely that both dicamba-resistant cotton and corn and soybeans being treated with dicamba will be grown in proximity, greatly increasing the total acres exposed to dicamba in a given year. And because of cotton and corn or soybean rotations, the likelihood that dicamba will be used on the same acreage one year after the next is greater as well.

At a landscape level this change in dicamba use pattern will result in a larger number of individuals of a wider array of species in proximity to MON 88701 cotton fields and thus to dicamba, with attendant impacts.

Also, levels of dicamba in water bodies within particular watersheds are likely to increase with more extensive use, as dicamba loads from different fields run off and mix together in creeks and rivers draining the watershed (Thurman et al. 2001).

These impacts of more extensive use of dicamba with MON 88701 cotton need to be assessed by EPA, in addition to other impacts.

4) Impacts to biodiversity of dicamba use with MON 88701 cotton

EPA needs to fully consider the impacts to biodiversity of the substantial changes in herbicide use amounts and patterns that are part of the MON 88701 cotton system, based on the full rates and maximum number of applications allowed by label, and extent of use in a given year and across years.

a. Biodiversity in cotton 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 crops, major impacts of associated herbicide use are just now surfacing, with only a handful of researchers doing this kind of "post-market" ecological research. EPA needs to consider these kinds of harms, and how to prevent them, before rather than after registering dicamba for new use with MON 88701 cotton.

The use of dicamba with MON 88701 cotton will result in more applications per season of dicamba, also a systemic herbicide, and likely to be used in addition to full rates of glufosinate (with which it is co-engineered for resistance, Brinker et al. 2012) and glyphosate (with which it is likely to be stacked for resistance, Voth et al. 2012). It is also reasonably foreseeable that in the future MON 88701 cotton will be treated with dicamba, glufosinate and glyphosate, in rotation with dicamba-resistant corn and dicamba-resistant soybeans 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 with MON 88701 cotton to plant population diversity within cotton 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 MON 88701 cotton tissues. A wide variety of animals feed on cotton leaves, flower parts, and seeds, including many beneficial organisms such as honeybees and other pollinators.

Also, some animals may be over-sprayed during applications of dicamba, and others may brush against newly sprayed foliage, receiving higher dicamba doses in MON 88701 cotton with possible toxic impacts (US-EPA 2009, Freemark and Boutin 1995). Animals at particular risk are discussed in our comments on listed species, below.

EPA needs to assess potential impacts to animals in fields of MON 88701 cotton in light of the foreseeable increase in exposure to dicamba and metabolites based on realistic use scenarios and a wide range of relevant independent scientific studies.

b. Biodiversity around cotton fields

Increased spray drift, volatilization and runoff from use of dicamba with the MON 88701 cotton is likely to alter the very habitats important for biodiversity in agroecosytems, such as hedgerows, riparian areas, unmanaged field margins, and other areas where wild organisms live near fields (Freemark and Boutin 1995, Boutin and Jobin 1998, Olszyk et al. 2004). Based on experiences with dicamba sensitive crops, natural areas miles from agricultural applications of dicamba may also be at increased risk from the use of greater amounts of the herbicide in MON 88701 cotton, since it can volatilize under certain conditions, as we have discussed earlier in these comments.

Particular species of plants are more or less sensitive to dicamba, and at different times of the year, so that a specific off-target dicamba event is likely to change the population dynamics in affected areas. For example, dicamba movement 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 dicamba 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 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 cotton fields. 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 dicamba may impact animals has to do with the ability of dicamba 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.

Herbicides such as dicamba that selectively kill dicots may be particularly injurious to butterflies, often considered an indicator of ecosystem health. If these herbicides are applied frequently and over a broad area – as will happen with dicamba use on MON 88701 cotton– negative impacts on butterflies are particularly strong. A study of pesticide effects on butterflies in agricultural areas of England makes this point:

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

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

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

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

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

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

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

Implications of this butterfly study in England are clear for use of dicamba with MON 88701 cotton: dicamba is an herbicide that selectively kills broadleaved plants (dicots), the main nectar source for adult butterflies, even those species whose larvae feed on grasses. Dicamba is also likely to be used more often during a season, more extensively in an area, and from year to year with MON 88701 cotton than it is currently used on cotton or in agriculture. This is exactly the opposite use pattern than that recommended for mitigation of pesticide impacts on butterflies, that were also shown to be protective of biodiversity in general.

These studies with butterflies are likely to indicate broader impacts. EPA provides a summary of dicamba impacts to plants with cascading effects for biodiversity:

> The guideline terrestrial plant studies indicate that dicamba negatively impacts seed germination (radicle length), seedling emergence (shoot length), and vegetative vigor (shoot weight) in monocots and dicots. Non-lethal effects included brown leaf tips, necrosis, decrease in size, leaf curling, chlorosis, and stem tumors. Dicamba acid and its'salts in formulated TEPs are readily absorbed through the foliage and roots of plants; consequently, it could be injurious to non-target plant species by drift, runoff, or leaching to roots. Damage to non-target plants may be sufficient to prevent the plant from competing successfully with other plants for resources and water. Listed plant species may be especially impacted by exposure to dicamba

> because of the impact of the loss of a few individuals to the population. Consequently, there is a potential concern for listed species with either broad or narrow dependencies on impacted plan species/populations/communities for habitat, feeding or cover requirements. In terrestrial and shallow-water aquatic communities, plants are the primary producers upon which the succeeding trophic levels depend. If the available plant material is impacted due to the effects of dicamba and the salts of dicamba, this may have negative effects not only on the herbivores, but throughout the food chain. Also, depending on the severity of impacts to the plant communities [i.e., forests, wetlands, ecotones (edge and riparian habitats)], community assemblages and ecosystem stability may be altered (i.e. reduced bird populations in edge habitats; reduced riparian vegetation resulting in increased light penetration and temperature in aquatic habitats, loss of cover and food for fish). (US EPA 2005, p. 75)

It is clear, then, that increased use of dicamba with the MON 88701 cotton is likely to have negative impacts on biodiversity around cotton fields, perhaps at some distance.

5) Impacts to threatened and endangered species of dicamba use with MON 88701 cotton

EPA needs to assess how the new use of dicamba with MON 88701 cotton will impact threatened and endangered species, based on the full rates and schedule that will be allowed by label, and extent of use.

All of the harms from increased use of dicamba, and applications later in the season, with MON 88701 cotton 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 where dicamba is applied to MON 88701 cotton will be at increased risk from exposure to dicamba 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 dicamba exposure are higher for listed species, 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).

EPA has determined in a preliminary risk assessment for listed species that "dicamba exceeds LOCs" for the following taxonomic groups, at all application rates considered (lowest rate 0.75 lb a.e./acre):

- small birds (20 and 100 g) feeding on short grasses, tall grasses, and broadleaf forage/small insects at all application rates and maximum and mean predicted residue levels

- small birds (20 g) feeding on fruit, pods, seeds/large insects at all application rates and maximum predicted residue levels

- large birds (1000 g) feeding on short grasses, tall grasses, and broadleaf forage/small insects at all application rates and maximum predicted residue levels

- non-target terrestrial plants - monocots and dicots adjacent to treated areas and in semiaquatic areas at all application rates (all uses modeled) by ground and aerial spray application; dicots in spray drift at all application rates (all uses modeled) by ground and aerial spray application (US EPA 2005) p. 73, excerpts for "all application rates" only.

Critical habitat is also clearly at risk from dicamba use given the potential for indirect effects via plants, which constitute the base of ecosystems. (US EPA 2005) p 75.

Summarizing these findings, EPA concludes:

> 4. Risk to Endangered Species
> The Agency's screening level ecological risk assessment for endangered species results in the determination that dicamba will have no direct acute effects on threatened and endangered freshwater fish, estuarine fish, and aquatic invertebrates. However, the assessment indicates that dicamba has the potential for causing risk to endangered birds, mammals, and non-target plants. Further, potential indirect effect to any species dependent upon a species that experiences effects cannot be precluded from use of dicamba. These findings are based solely on EPA's screening level assessment and do not constitute "may effect" findings under the Endangered Species Act. Chronic RQs exceeded LOCs for endangered mammals at all application rates modeled. Acute LOCs were exceeded for endangered birds at all application rates. LOCs were exceeded for terrestrial plants adjacent to treated areas and in semi-aquatic areas at all application rates. (US EPA 2006) p. 20.

EPA enacted environmental risk mitigation measures because of these non-target risks, lowering the maximum single application rate to 1.0 lb ae/acre, and the yearly rate to 2.0 lb ae/acre per year, but states that these measures will not eliminate risks: "This will result in lowering the potential risks of concern to aquatic plants. This rate reduction will also lower acute risks to all animals (except small herbivorous birds), as well as chronic risk to mammals. Assessed risks to terrestrial plants will be lowered, but not eliminated" (US EPA 2006, p. 23). In particular, risks to specific listed species from dicamba need to be addressed, and have not been to date, as far as CFS can determine.

However, 2,4-D – another auxin herbicide – was recently subjected to a Pesticide Effects Determination by EPA (US-EPA 2009) and Biological Opinion from the National Marine Fisheries Service (NMFS 2011), both finding adverse impacts of agricultural uses of 2,4-D to several specific endangered species. The detailed information in these reports can be extrapolated to mean that almost all threatened and endangered species would be similarly impacted by dicamba with MON 88701 cotton.

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 dicamba on MON 88701 cotton would similarly be "likely to adversely affect" prey and habitats of threatened and endangered animals found near these fields, given the similarities between 2,4-D and dicamba in many respects. In particular, the ability of 2,4-D to alter habitat by injuring plants, and thus changing plant populations is relevant to the potential for dicamba to do the same.

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).

Although cotton is not grown in the Pacific Northwest, many threatened and endangered aquatic species will have similar habitat requirements for water quality and prey as do Pacific salmonids, including some that are in habitats near cotton cultivation and thus could be impacted by the increased use of dicamba on MON 88701 cotton.

EPA did produce a brief "no effect determination" for dicamba and the Pacific salmonids in 2003 (US EPA 2003), but it only considers direct toxicity impacts of dicamba on aquatic organisms, including the fish themselves. It does not consider any of the kinds of impacts from dicamba injury to plants or to habitat found to be problematic for 2,4-D. Given the results of NMFS' biological opinion where these habitat effects were found to be significant, EPA needs to reconsider their limited "no effect determination" for dicamba.

In fact, because of the determinations regarding 2,4-D and CRLF, AW and Pacific salmonids, combined with scientific studies on impacts of herbicides on biodiversity, and the information on risks to listed species in dicamba RED, EPA should initiate consultations with FWS and NMFS concerning the use of dicamba with MON 88701 cotton. EPA's consultation duties under the ESA on the direct and indirect impacts of its approval action in no way vitiates the ESA duties of any other agencies (such as USDA/APHIS) for the impacts of their own approval action.

VI. Conclusion

Clearly, the proposed registration for use of dicamba on MON 88701 cotton would have numerous serious and adverse impacts, as discussed in detail above.

CFS would be happy to discuss the issues raised in these comments with EPA staff in the interests of a full, rigorous and scientifically credible assessment of the proposed registration for use of dicamba on MON 88701 cotton.

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