December 6, 2010

Docket No. APHIS-2010-0047
Regulatory Analysis and Development
PPD, APHIS
Station 3A-03.8
4700 River Road Unit 118
Riverdale, MD 20737-1238

RE: Comments on APHIS Docket No. APHIS-2010-0047
Draft Environmental Assessment: Monsanto Company and KWS SAAT AG Supplemental Request for Partial Deregulation of Sugar Beet Genetically Engineered to be Tolerant to the Herbicide Glyphosate

Center for Food Safety - Science Comments II:
Human health and environmental consequences of increased glyphosate use with H7-1 sugar beets

Glyphosate use will increase under Alternatives 2 or 3.

Alternative 1 "would deny the request for 'partial deregulation'…of event H7-1 sugar beets, thereby halting any consideration of authorizing commercial production until the completion of the EIS" (Draft Environmental Assessment, DEA, p. 4).

Glyphosate use would be much less with conventional sugar beets grown under Alternative 1 in comparison to glyphosate use with H7-1 sugar beets under Alternatives 2 or 3. For example, before adoption of H7-1 sugar beets in 2000, the average amount of glyphosate used on sugar beets was 0.43 lb/acre in 1.1 applications per year on 13% of sugar beet acres. Total glyphosate used in 2000 on sugar beets was 86,000 pounds (DEA p. 78, Table 3). After H7-1 adoption, by 2010 that amount increased to 1.5 – 2.25 lbs/acre in 2-3 applications and covered 95% of sugar beet acres. Total glyphosate used on sugar beets in 2010 was about 1.975 million pounds, 23X higher than ten years earlier (DEA p. 85, Table 6). Glyphosate use per acre is likely to increase further as weed control with glyphosate becomes more difficult due to resistant and tolerant weeds.

Under Alternatives 2 or 3, the total amount of glyphosate would increase per acre, and the window of glyphosate use during the season would widen. Instead of a single glyphosate application occurring mainly for pre-planting burndown, glyhposate would be applied an additional one or more times during the growing season and, thus, potentially come into contact with different animals, plants, and microorganisms at different stages in their lifecycles, resulting in more detrimental impacts to eco-sytems.

APHIS did not take into account recent studies showing adverse effects of glyphosate.

APHIS claims that the increased glyphosate use likely to accompany planting of H7-1 sugar beets is a benefit to human health (for example, DEA p. 222) and the environment to the extent that glyphosate replaces other herbicides commonly used with sugar beets that are considered to be more toxic. They also argue that glyphosate use with H7-1 sugar beets will lead to environmental benefits from changes in farming practices, such as greater adoption of conservation tillage.

These claims are valid only if the health and environmental consequences of glyphosate use have been carefully evaluated, and then to the extent that glyphosate does replace other more toxic herbicides.

Glyphosate impacts should be considered separately from other herbicides.

Use of glyphosate is tied to the Roundup Ready system and will be applied to all H7-1 acres, so analysis of the consequences of increased glyphosate use is integral to the DEA. Increased glyphosate use should be evaluated independently of the herbicides it might replace. If it is found to have negative human health and environmental consequences, these should be considered in the DEA irrespective of risks of other herbicides.

On the other hand, the kinds and amounts of other herbicides used on conventional sugar beets are not proscribed by that system and can be changed by growers in response to health and environmental concerns. For example, APHIS states that "[c]urrent sugar beet herbicide products containing triflusulfuron, trifluralin and pyrazon are shown to exceed these Levels of Concern" for toxicity to aquatic organisms (DEA p. 165 - 166). Although APHIS includes trifusulfuron as exceeding the Level of Concern in the text, the tables showing toxicity (Tables 12 and 13) do not indicate that it does trigger concern. However, trifluralin and pyrazon do, and were only applied on 5% and 6% of sugar beet acres in 2000 (DEA p. 78, Table 3), so could presumably be discontinued without too much disruption to growers.

APHIS based calculations of health and environmental benefits on the use of glyphosate rather than a suite of other herbicides. These calculations assume that glyphosate will be the only weed control method used with H7-1 sugar beets. Given the history of glyphosate use on other Roundup Ready crops, assuming exclusive use of glyphosate is reasonable for the first few years. However, APHIS also claims that H7-1 farmers will use good weed resistance management methods, and part of good weed resistance management is the use of other tools in addition to glyphosate including herbicides with other modes of action, tillage, and hoeing. What mix of weed control practices will be used by farmers practicing good weed resistance management for H1-7 sugar beets, and how will calculations of human health and environmental consequences be adjusted for these mixed practices?

For example, in analyzing the consequences of using less glyphosate and more of other herbicides under Alternative 1, APHIS relied upon life-cycle assessment calculations to predict that "[e]missions related to global warming, ozone depletion, summer smog and carcinogenicity, among others, were found to be lower in glyphosate-tolerant crop systems compared to conventional crop systems (Bennett et al., 2004)." These researchers assumed that glyphosate would only be applied twice post-emergence, and that no other weed control methods would be used (Bennett et al. 2004, Table 1). They stressed that results and possibly rankings of herbicides would change with assumptions: "Clearly, these results are dependent on the number of herbicide spray applications under each growing system, as well as the nature of the herbicides applied. Indeed, sensitivity analyses revealed that assumptions concerning the number of applications and the amount of herbicide applied to the crop were most important in affecting a number of impacts." (Bennett et al. 2004, p. 276) There were also serious data limitations with this analysis: "Guinee (2002) notes that, for any LCA, 'in practice, data are frequently obsolete, incomparable or of unknown quality'." Finally, the relative life-cycle impacts of different herbicides are likely to vary with particular growing sites and seasons, rather than be fixed at all locations. Therefore, the environmental advantages calculated for glyphosate over other herbicides may not hold in real-world situations.

<u>APHIS overlooked peer-reviewed studies showing endocrine disrupting properties of glyphosate.</u>

Starting with human health concerns related to glyphosate, APHIS overlooked recent peer-reviewed studies showing potentially harmful consequences of using glyphosate-based herbicides, and thus underestimated the possible impacts of deregulating H7-1 sugar beets.

The studies raising health concerns implicate glyphosate-based herbicides in disruption of endocrine systems. Endocrine-disrupting chemicals interfere with the action of hormones, and usually function at very low concentrations, often approximating environmental exposure levels. Depending on the hormones affected, the health consequences of disruption range from birth defects to propensity for diseases later in life. Some effects of endocrine disruption are experienced by subsequent generations (Colborn and Carroll 2007).

Pesticides, including glyphosate, have not been subjected to low-dose testing specific for endocrine disruption as part of their regulatory approval. For example, the review of health studies on glyphosate by Williams et al. (2000) summarizes dozens of studies in which glyphosate is fed to various animal species in acute and chronic toxicology experiments. The lowest dose tested in any study was 3 mg glyphosate/kg body weight/ day, and this was unusual (Williams et al. 2000, p. 127). Most studies started much higher concentrations.

Results from high-dose studies cannot simply be extrapolated to low doses. According to Meyers et al. (2009), "[t]he core assumption of regulatory toxicology is that experiments using high doses will reveal potential effects of low doses…although it conflicts directly with well-established principles in endocrinology regarding hormone actions." They say that "[i]ndeed, Paracelsus' assumption [that "the dose makes the poison"] is directly contradicted by decades of research in endocrinology and clinical medicine showing that hormonally active compounds have dose-response curves in which low doses can cause effects opposite to those at high doses." An example is the stimulation of breast cancer cells by tomoxifen at low doses, but suppression at high doses. Low doses can also cause qualitatively different effects through binding to different receptors or bypassing the receptor system, causing different genes to turn up or down, and so on.

Another example is the different vegetative growth response of plants to glyphosate at high and low doses. At field rates used to kill weeds, glyphosate causes stunting. But at low drift rates (more than 10X lower than typical field rates) glyphosate can stimulate shoot growth, perhaps by affecting levels of hormones (Cedergreen 2008; Velini et al. 2010).

In a review of pesticides and animal reproduction, Colborn and Carroll (2007) point out the inadequacies of current regulatory testing for determining endocrine disruption:

> "The outmoded 'Maximum Tolerated Dose' or the U.S. Agency for Toxic Substances and Disease Registry's (ATSDR's) Minimum Risk Levels (MRLs) are based on crude, traditional toxicological protocols and endpoints that have almost completely missed low-dose, endocrine system-mediated effects." (p. 1103)
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> "However, when testing synthetic chemicals for regulatory purposes, traditional testing has not focused on testing at ambient exposure concentrations nor taken into consideration the extremely low concentrations of naturally produced chemicals that control the endocrine system….To date, pesticide toxicological testing requirements, risk assessment models, and exposure standards have not seriously nor rigorously responded to the threat posed by endocrine disruptors. Despite passage of the U.S. Food Quality Protection Act (FQPA) in 1996 and its clear mandate and provisions calling for the testing of pesticides for their potential to impair endocrine system function, the U.S. Environmental Protection Agency (USEPA) has yet to develop and adopt a series of standardized assays to meet the mandate. Consequently, pesticides continue to be registered and reregistered based on traditional toxicological assays, ignoring the growing evidence in the open literature that some widely dispersed pesticides interfere with development and function at ambient exposure concentrations." (p. 1079)

The USEPA is beginning the process of testing all pesticides for endocrine disruption and glyphosate is on the schedule (Status of the USEPA's Endocrine Disruptor Screening Program, EDSP; http://www.epa.gov/endo/).

The EDSP requires pesticide registrants to subject their products to a tiered testing procedure. The first tier of tests includes a battery of assays to detect chemicals that interfere with endocrine functions. EDSP Tier 1 assays effects of pesticides on frog metamorphosis, androgen receptor binding (rat prostate cytosol), aromatase (human recombinant), estrogen receptor binding (rat uterine cytosol), estrogen receptor transcriptional activation (human cell line HeLa-9903), fish short-term reproduction, Hersberger (rat), female pubertal (rat), male pubertal (rat), steroidogenesis (human cell line, H295R), and uterotrophic (rat) (http://www.epa.gov/ocspp/pubs/frs/publications/Test_Guidelines/series890.htm). Tier II tests are still being finalized, but drafts include amphibian development and reproduction over multiple generations, resulting in a risk assessment.

These low-dose studies specifically designed to look for effects on hormone systems in a variety of animal species and cell types are considered to be diagnostic for human health outcomes. According to Meyers et al. (2009), "Because the endocrine system is highly conserved between animals used as models in biochemical research and humans, the default assumption should be that nonmonotonic [different low and high] dose-responses of EDCs [endocrine disrupting chemicals] observed in laboratory animals and *in vitro*, including with human cells and tissues, are applicable to human health…".

Although the USEPA has not tested glyphosate yet, independent researchers around the world have been subjecting glyphosate to endocrine-disruption assays for many years. Some of the tests, using assay systems similar to those chosen by the USEPA, have implicated glyphosate and/or it formulations.

A recent peer-reviewed study from Argentina provides evidence in favor of glyphosate as an endocrine disruptor. Paganelli et al. (2010) used tadpoles of the African Clawed Frog, *Xenopus laevis,* to test whether glyphosate, both formulated as "Roundup Classic" and as active ingredient alone, caused abnormal development. *Xenopus laevis* is a well-established model system for embryo development, and hormonal responses are similar in frogs and humans. Paganelli et al. examined development along the anterior/posterior axis. At the molecular level, several pathways involving a host of signals and transcription factors must be coordinated to produce a normal head-to-tail organism, so this is a sensitive system for studying perturbations.

To determine if glyphosate would disrupt tadpole development they incubated developing frog embryos in Roundup Classic at dilutions of 1/3000, 1/4000, and 1/5000 (lowest concentration 71 mg a.e./liter or 421 uM glyphosate). At all of these dilutions, they observed abnormal development. Therefore, they did not use low enough concentrations to establish a no-effect level.

Glyphosate salt alone injected into one side of developing embryos at a final concentration of 1.35 – 2.02 mg a.e./liter or 8 – 12 uM glyphosate resulted in similar abnormalities: "marked alterations in cephalic and neural crest development and shortening of the anterior-posterior (A-P) axis." [Abstract] The researchers also looked at expression of marker genes that are normally expressed in the brain and head neural crest. Glyphosate treatments resulted in lower expression of all the marker genes, and only on the side of the embryo that was injected.

The defective phenotype was a tadpole with a smaller head and face but the rest of the tadpole looked normal, so they wanted to narrow down the developmental genes being affected. A signal molecule called "sonic hedgehog" (Shh) is normally secreted by the floor of the central nervous system and spreads from there to pattern the head and face. Blocking Shh results in a shrunken head and face. Glyphosate induced the same kinds of defects (Fig 2 and 3), suggesting that it interfered with Shh signaling. They showed that glyphosate did reduce Shh signaling and the effect was dose dependent (Fig4).

Retinoic acid (RA) is involved in head formation, and there is a gradient of RA in embryos that is high at the tail and low at the head. When RA levels are low, Shh is expressed, and the tadpole makes a normal head. Paginelli et al. hypothesized that glyphosate mimicked the effects of RA by binding to an RA receptor. If so, there should be an increase in expression of genes that respond to RA when embryos are treated with glyphosate – resulting in no head - that can be counteracted by simultaneously treating embryos with glyphosate and a molecule that blocks the RA receptor. When they added the RA blocker with glyphosate, a normal head formed.

Because the RA/Shh anterior forming is characteristic of vertebrates in general, glyphosate should have a similar effect on other vertebrate species. They show similar results in chick embryos, but not at the same level of detail and without controls.

These experiments showing that glyphosate and glyphosate-based formulations can disrupt early vertebrate development via a well-known, specific genetic pathway need to be extended to include lower concentration ranges in order to determine risks to humans and wildlife. However, the specificity of the glyphosate effect in frog development makes this a strong result.

Another approach for determining endocrine effects of glyphosate and glyphsoate-based herbicides is to test their ability to cause hormone-dependent cells to divide in culture. Lin and Garry (2000) at the University of Minnesota studied estrogenic effects of herbicides, fungicides and adjuvants commonly used in the Red River Valley region of Minnesota (a sugar beet growing area), using a human breast cancer cell line, MCF-7, to assay the chemicals. Glyphosate (reagent grade isopropylamine salt) did induce statistically significant cell proliferation at 10 to 100 uM concentrations (.228 – 2.28 mg/l) as did 2,4-D and two of the five adjuvants tested.

Another test required by USEPA to indicate endocrine disruption is for steroidogenesis, the biochemical pathway leading to synthesis of testosterone, estrogen and other steroid hormones, using human cell line H295R that originated from an adrenocortical tumor (EPA 640-C-09-033, 2009).

Walsh et al. (2000) at Texas Tech University in Lubbock used a different steroidogenesis test system, the mouse MA-10 Leydig tumor cell line from testes, and a regulatory protein for the rate-limiting step in steroidogenesis. They found that 25 mg/l (~150 uM glyphosate) of Roundup inhibited steroidogenisis by disrupting the expression of this key protein, without decreasing total protein synthesis. Glyphosate alone did not have any effect, nor did any of the other 7 pesticides tested.

Aromatase activity is another endocrine disruption test system. The USEPA has chosen a human recombinant enzyme assay, "… intended to identify chemicals that may affect the endocrine system (*e.g.*, steroidogenesis) by inhibiting catalytic activity of aromatase, the enzyme responsible for the conversion of androgens to estrogens." (EPA 740-C-09-004, 2009)

Researchers in France used cultured human placental JEG3 cells to assess effects of Roundup and glyphosate on the aromatase system (Richard et al. 2009). They found that Roundup was more effective than glyphosate alone at inhibiting aromatase activity, and concluded that the formulation had endocrine disrupting activity.

Fish have exhibited endocrine responses to glyphosate, as well. The most recent study was by Jaensson (2010). Glyphosate (active ingredient), in common with some other pesticides tested, affected the endocrine system of male Atlantic salmon by suppressing 11-ketotestosterone levels (11KT). She concluded:

> "It appears the synthesis of 11-KT in the salmonid parr is affected by pesticide interference with the olfactory and endocrine system. Production of 11-KT was significantly altered by exposure to cypermethrin and glyphosate, both during priming and behavioural studies. Cypermethrin appears to decrease reproductive behavioural responses as well as sex hormone level implying that there may be an effect in the olfactory system. The effects described from copper exposure seem to be from mechanisms acting upon both the olfactory system and the endocrine system as there was a reduction in spawning behaviour and milt production, but not in sex hormones. However, glyphosate did not causes observable behavioural changes and only lowered 11-KT indicating that any effects were acting directly upon the endocrine system and not via olfactory regulation.
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> "Our results demonstrate that there is a possibility of environmentally relevant pesticide concentrations being capable of suppressing the brown trout and Atlantic salmon male endocrine response, including spawning behaviour, to female pheromones. Detected levels in natural water ways of …glyphosate can range from 328 µg L-1 to 0.02 µg L- 1(Scribner, et al. 2003; Battaglin et al. 2008; Struger et al. 2008). The levels of exposure in the current study are, therefore environmentally relevant (… glyphosate 150 µg L-1). Investigating the effects of relevant levels of exposure is imperative to understanding what is happening in the environment. It is seldom that these animals actually come in contact with acute levels of pesticides. However, their environments are inundated with low levels of pesticides, and more research will need to be done using environmentally relevant exposures."

<u>APHIS did not evaluate differences between impacts of formulations and active ingredient.</u>

Results from various labs differ in the degree to which formulations versus glyphosate itself interact with endocrine system parameters, which is not surprising. Studies using commercial formulations are difficult to compare because adjuvant mixes differ over time and between brands. Often researchers are unable to find out what components are present because of trade secret protections. Therefore, they are seldom able to include formulation minus active ingredient controls in experiments.

Difficulties notwithstanding, testing formulations for health and environmental consequences is important. Most tests done for regulatory approval involve the active ingredient alone, or include studies on one common surfactant alone (Cox and Surgan 2006, Williams et al. 2000). According to Cox and Surgan (2006), testing the active ingredient is insufficient for gauging toxicity of a formulation. In some cases, increased toxicity in formulations results from interactions between the active ingredient and adjuvants, in other cases increased toxicity is primarily due to adjuvants alone. They conclude that inert "ingredients can increase the ability of pesticide formulations to affect significant toxicological end points, including developmental neurotoxicity, genotoxicity, and disruption of hormone function. They can also increase exposure by increasing dermal absorption, decreasing the efficacy of protective clothing, and increasing environmental mobility and persistence. Inert ingredients can increase the phytotoxicity of pesticide formulations as well as the toxicity to fish, amphibians, and microorganisms."

Epidemiological studies, where populations exposed to glyphosate are compared to similar populations that had lower or no exposure, can sometimes show health effects of glyhposate-based herbicide use as it happens in the field. An epidemiological study was analyzed by De Roos et al. (2005a) at the prestigious Fred Hutchison Cancer Research Center in Seattle, and they looked at cancer incidence data from the Agricultural Health Study (AHS). This was a "prospective cohort study of 57,311 licensed pesticide applicators in Iowa and North Carolina." The applicators filled out detailed pesticide use questionnaires when they enrolled in the study, from 1993 – 1997 (before the big increase in glyphosate use due to Roundup Ready cropping systems). Then the researchers waited for cancers to appear. They concluded that there was no evidence of association between glyphosate and 11 fairly common cancer types, but that there was a possible association between glyphosate and the risk of multiple myeloma. Their statistical analyses supported the link, but sample sizes were small, so they suggested doing follow-up studies as the years go by and more cancers continue to appear. They said that follow-ups are important "[g]iven the widespread use of glyphosate, future analyses of the AHS will allow further examination of long-term health effects."

In spite of De Roos et al. adopting a cautious interpretation of their data, their study was criticized by Monsanto's Farmer et al. (2005) for a variety of shortcomings, including for ignoring extensive reviews of glyphosate toxicology in Williams et al. (2000) that showed no cancer increases or genotoxicity predictive of cancer, even at very high doses. De Roos et al. (2005b) responded by pointing out that the studies showing "low toxicity" were based on glyphosate alone, not formulations. They also argued that the "dose thresholds Farmer et al. cite as relevant for carcinogenicity are from mouse and rat models in which the active ingredient, glyphosate, was tested in feeding studies (Williams et al. 2000). Lower relevant doses may apply for Roundup and other formulated products used in combination with other active ingredients. In addition, epidemiology can provide direct information on the question of what happens in humans from more relevant routes of exposure." De Roos et al.

reiterate that the "most reliable approach [to determining if multiple myeloma is a risk from glyphosate] will be to reanalyze the data after more cases accumulate….".

In summary, APHIS did not take into account results from these and other studies showing effects of glyphosate on endocrine functions, and possibly as a risk factor for multiple myeloma, when they determined that increased glyphosate use accompanying planting of H7-1 sugar beets is better for human health than the alternative of growing conventional sugar beets. Therefore, the purported health benefits of using more glyphosate may not exist.

Environmental consequences of increased glyphosate use need to be taken more seriously.

APHIS asserts that an increase in glyphosate use will be less harmful to the environment in general than the herbicides used instead if Alternative 1 is adopted. For example, under "Environmental Consequences: Biological Resources: Weed Management" (pp. 164 – 166), APHIS says that "several of the herbicides used in conventional sugar beet systems may have more adverse impacts than glyhosate on aquatic and terrestrial plant species…", and that "neighboring crops and plants at risk from the use of glyphosate are also potentially at risk from the use of any other herbicide" but that glyphosate binds to soil and is used in no-till practices so is less likely to end up in water. They also state that "[h]undreds of millions of acres of other glyphosate tolerant crops have been treated with glyphosate for over ten years with minimal impact to adjacent non-target terrestrial plants." No studies are cited in support of this assertion regarding non-target plants.

APHIS did not seriously consider off-site movement of glyphosate via soil and water that could impact wild animals and plants.

APHIS does not adequately consider the possibility that glyphosate will move off-site via soil and water. They stress the tight binding of glyphosate to soil particles, on the one hand, and its rapid degradation by bacteria in soil on the other hand. They also state that conservation tillage, increased by RR crop adoption, will further reduce the movement of glyphosate off-site via soil-particle-laden water runoff. Thus, they conclude that wild animals and plants outside of sugar beet fields will have little contact with glyphosate via soil and water. Specifically, APHIS does not adequately consider the many studies (recently reviewed by Borggaard and Gimsing 2007) concluding that glyphosate behavior in soil and water is quite varied, depending on soil type and structure, weather conditions before and after glyphosate application, extent of vegetation, previous management of the land (amount of phosphate fertilization, for example), water table, and other factors.

In 2002, the USGS began a monitoring program for glyphosate and its main degradation product, AMPA, in Midwestern streams (Battaglin et al. 2005), because "…the use of glyphosate is increasing rapidly, and there is limited understanding of its environmental fate". Glyphosate was detected in about a third of the streams sampled, after rain events following pre-emergence, post-emergence and harvest seasons. The highest measurement in these streams was 8.7 ug/l in a harvest-season sample.

The authors encouraged further study of glyphosate in water:

> "However, it also appears that glyphosate and AMPA are more mobile or persistent in aquatic environments than earlier research and monitoring suggested (Giesy et al., 2000). Additional monitoring for glyphosate to include summer low flow and wintertime samples could provide the information needed to determine which use, fate, and transport factors have the most influence on their environmental occurrence. Additional monitoring will be needed to determine if the increasing use of glyphosate results in increasing glyphosate and AMPA concentrations in Midwestern streams."

In follow-up studies, Battaglin and his colleagues observed vernal pools and streams near herbicide application sites in National Parks (Battaglin et al. 2009):

> "Abstract. Vernal pools are sensitive environments that provide critical habitat for many species, including amphibians. These small water bodies are not always protected by pesticide label requirements for no-spray buffer zones, and the occurrence of pesticides in them is poorly documented… Glyphosate was measured at the highest concentration (328 ug/l) in a sample from Riley Spring Pond in Rock Creek National Park. This concentration exceeded the freshwater aquatic life standard for glyphosate of 65 ug/l."

Some of the concentrations in these vernal pools were high enough to be toxic to amphibians, assuming that the glyhposate levels reflect application of common formulations.

<u>APHIS did not review the known effects of glyphosate on amphibians</u>**.**

Of animals tested so far, amphibians of many species are more sensitive to glyphosate-based herbicides than other taxa (Relyea 2005a, 2005b, 2006; Relyea and Jones 2009; Bernal et al. 2009a, 2009b). Many species have been tested around the world, and none of the tests were cited by APHIS. Amphibians are poisoned by glyphosate-containing herbicides at larval and adult stages, so could be exposed via water or contact with sprayed foliage. They could also ingest insects that had eaten glyphosate-treated plants, and thus be exposed via food.

On average, most of the studies of glyphosate-based herbicide toxicity to larval amphibians found that half of them died within 4 days at between 0.8 and 3.2 mg a.e./liter of glyphosate. Formulations with different adjuvants gave similar results. To protect most larval amphibians from harm, the concentration of glyphosate would have to be much lower, by an order of magnitude, which would be in the range of concentrations found in some vernal pools (Relyea 2006, Battaglin et al. 2009).

Adult or terrestrial frogs that are directly over-sprayed have LC50 4-day values of 4.5 to 22.8 kg a.e./ha (Bernal et al. 2009b), which means that spray rates must be much lower than that to protect most frogs from harm. If the amounts were lowered by an order of magnitude to provide a margin of safety, the allowed spray rates for H7-1 sugar beets, 0.86 – 1.26 kg a.e./ha (DEA,p. 84), would be in the toxic range.

Here is what APHIS had to say about impacts of herbicides in sugar beet on amphibians (pp. 99 – 100, Affected Environment: Biological Resources: Animals):

> "Amphibians and Fish. Amphibians and fish are not directly impacted by the growth of the sugar beet plant in that they do not use sugar beet for food or shelter. Indirectly, the herbicides currently used on conventional sugar beets can affect amphibians and fish in water bodies adjacent to or downstream from, the treated field, and might include impounded bodies such as ponds, lakes and reservoirs, or flowing waterways such as streams or rivers."

As just discussed, Battaglin et al. (2009) showed that water bodies adjacent to glyphosate-treated fields can also be contaminated to the level of toxicity for amphibians, but APHIS only considered that other herbicides would do that.

For effects on endangered amphibians (pp. 232 – 233, Environmental Consequences: Threatened and Endangered Species):

> "As a part of EPA's TES effects assessment for the California red-legged frog (US EPA, 2008), EPA evaluated the effect of glyphosate use at rates up to 7.95 lb a.e./A on fish, amphibians, aquatic invertebrates, aquatic plants, birds, mammals, and terrestrial invertebrates. This assessment determined that at the maximum application rate for in-crop applications of glyphosate to GT sugar beets (1.125 lb a.e. /A) there would be no effects of glyphosate use on the following taxa of threatened and endangered species: fish, amphibians, birds, and mammals. The EPA assessment was uncertain of the effects on terrestrial invertebrates, citing the potential to affect small insects at all application rates and large insects at the higher application rates."

There were no studies cited for the claim that amphibians do not use sugar beet fields for food or shelter. APHIS does say that snakes and rodents use sugar beet fields for food or shelter, also without studies, so why does APHIS analysis exclude toads or other amphibians from their natural environment? Furthermore, if insects are affected, amphibians that feed on insects could be harmed via secondary effects, and these impacts are not discussed.

Finally, APHIS stresses that many glyphosate-based herbicide formulations are not approved for use over water, but many studies have now shown that glyphosate gets into water bodies via known and unknown routes even when it is being used according to directions, and that vernal pools, so important for amphibian reproduction, often escape the regulations.

For these many reasons, the omission of amphibians from analysis of consequences of increased glyphosate use must be remedied.

<u>APHIS did not take into account possible negative consequences of increased glyphosate use on non-target plants.</u>

Plants are the targets of glyphosate the herbicide, so non-target plants are inherently at risk from inadvertent exposure. As already pointed out, APHIS states that "[h]undreds of millions of acres of other glyphosate tolerant crops have been treated with glyphosate for over ten years with minimal impact to adjacent non-target terrestrial plants." No studies are cited in support of this assertion regarding non-target plants.

In fact, studies of glyphosate drift exposure and effects would be informative because there are well-known sub-lethal effects of glyphosate on plants populations where glyphosate use has increased due to adoption of Roundup Ready cropping systems.

The data regarding sub-lethal glyphosate effects on plant reproductive success is reviewed, and added to by original research, in a key peer-reviewed scientific paper, not cited in the DEA (Blackburn and Boutin 2003).

This research specifically addresses risk to the success of wild plants from drift levels of glyphosate related to increased use of glyphosate associated with Roundup Ready crops. The authors state:

> "The use of these new crops has raised concern about an increase in reliance on glyphosate for weed control with detrimental consequences on nontarget plants and habitats due largely to the broad spectrum nature of this herbicide. The objective of this paper is twofold: (1) to review the literature on the effect of glyphosate on seed germination and early seedling growth, and (2) to present the results of a new experiment with several crop and noncrop species. The attempt was made to build on past findings and to add to the knowledge base in an effort to move away from studies on crop plants such as soybean and grain by focusing mainly on noncrop plant species." (Blackburn and Boutin 2003, p. 272)

Past findings are that sometimes plants that have not suffered mortality after contact with glyphosate, and in fact may not have exhibited visible symptoms of injury at all, nevertheless produce fewer seeds or seeds that have problems with germination or vigor (references cited in Blackburn and Boutin 2003). Also, plants that reproduce vegetatively from tubers or rhizomes sometimes show injury in the generation subsequent to actual glyphosate application or contact.

Specific, unique properties of glyphosate explain how it can affect subsequent plant generations. Glyphosate applied to leaves and stems translocates with photosynthates to the most rapidly growing tissues and organs of plants, such as developing flowers and seeds. In most plant species, including sugar beets, glyphosate is not metabolized, and these plant parts not only accumulate the glyphosate but also are particularly sensitive to it.

Therefore, glyphosate can cause pollen sterility, potentially resulting in fewer seeds; or can cause seeds that form to be less viable and vigorous. Different species of plants are more or less sensitive to glyphosate's sexual and vegetative reproductive effects, and the stage of development at which the plant is exposed to glyphosate influences the response, as well (Blackburn and Boutin 2003). In many cases, drift levels of glyphosate have been shown to cause these effects (Blackburn and Boutin 2003). Thus, sub-lethal doses of glyphosate can reduce the fitness of an affected plant species, reducing population levels in subsequent generations. Because other herbicides have different basic properties – for example, less efficient or no translocation to reproductive tissues, or metabolism within the plant resulting in less accumulation and persistence – the substitution of other herbicides by glyphosate is likely to have unique effects on plants, and preferentially affecting reproductive success at low rates may be one of these

Another factor that has not been studied is the impact of being able to apply glyphosate during the entire H7-1 sugar beet growing season. It is certain that wild plants will have the potential to be exposed to glyphosate during more of their growth phases, including closer to or during reproduction, making them more vulnerable to adverse reproductive outcomes. In their discussion, Blackburn and Boutin say this:

> "It is difficult to predict how glyphosate exposure will change a plant community due mainly to the wide variation in maturation and growth patterns of the species present at the time of application. Noncrop species growing within crops or along field margins where they may be exposed to glyphosate through overspray or spray drift may be at different phonological stages that the crop in which or near which they are growing (Shuma et al.,1995). Some species may have mature seeds while others may have immature seeds and still others may not be reproducing yet. The future of the seeds could be affected, while in the cases where plants are not at a stage of reproduction, the death or declined vigour of the plant could result in no input of seeds for the next generation. Herbicide applications may reduce the production of viable seeds and thus reduce the establishment or replenishment of noncrop seed reserves in the soil (Baskin and Baskin, 1998)."

Again, adoption of H7-1 sugar beets will increase the likelihood that many stages of a particular plant will be contacted by glyphosate, causing a more varied and perhaps cryptic injury pattern, not seen until the next generation. This type of injury will be difficult to monitor and mitigate given its cryptic nature.

There has been some recent interest in non-herbicidal effects of glyphosate that may operate at drift levels, too (Duke and Dayan 2010). These include changes in biochemistry (for example, sugar increases in cane), and growth enhancement (not to be construed as an advantage of drift – not all growth increases are adaptive).

If threatened or endangered plants are found near H7-1 sugar beet fields, farmers are supposed to take special measures as suggested by Monsanto (DEA pp. 232-233). Again, however, given the cryptic nature of sub-lethal glyphosate effects, such measures may not be adequate to protect the plants, even if the farmers do know that there are such species nearby and go to and follow the instructions on the Monsanto Pre-Serve website. In other words, the "legal precautions" represented by the EPA label use restrictions, may not be adequate given new knowledge about glyphosate effects on non-target plants.

<u>APHIS did not consider relevant research showing changes in rhizosphere microorganisms that are specific for Roundup Ready crop systems where glyphosate is used post-emergence.</u>

Under "Environmental Consequences: Biological Resources: Microorganisms", APHIS continues the general argument that under Alternative 1:

> "[T]he potential for impacts to microbial species may be greater than with Alternative 2 because of the return to greater use of additional herbicides, potentially with higher toxicities.
>
> Alternatives 2 and 3. Alternatives 2 and 3 are expected to result in continued glyphosate exposure to microbial species within and adjacent to those sugar beet fields through drift and direct and would likely decrease in exposure to other herbicides (USDA APHIS, 2009).
>
> Microorganisms produce aromatic amino acids through the shikimate pathway, similar to plants. Since glyphosate inhibits this pathway, it could be expected that glyphosate would be toxic to microorganisms. However, field studies show that glyphosate has little effect on soil microorganisms, and, in some cases, field studies have shown an increase in microbial activity due to the presence of glyphosate (USDA FS, 2003)."(DEA p. 189)

However, APHIS does not take into account the many studies showing that glyphosate does cause changes in populations of microorganisms in the rhizosphere of plants, both Roundup Ready and not, that have been treated with glyphosate and have translocated it to their roots where it can come into contact with specific microorganisms there. The studies showing "little effect" are almost all done by applying glyphosate directly to soil where it binds in the top few centimeters, and/or studies looking at gross markers of microorganism activity rather than specific functions or strains.

Also, glyphosate can inhibit some microorganisms and stimulate others, including pathogenic strains. Monsanto has done a lot of research using glyphosate as an antifungal chemical against rusts and other pathogens. Monsanto scientists presented information at a conference that the anti-pathogen activity of glyphosate is based on its systemic concentration and ability to inhibit the EPSPS enzyme. In other words, this research explains glyphosate's ability to translocate throughout the plant and then come into contact with the pathogens, inhibiting them in the same way it does plants (Duke and Dayan 2010).

Independent researchers have shown that glyphosate can also stimulate certain plant pathogens in the rhizosphere of Roundup Ready crops, where glyphosate within or being exuded in close proximity to the microorganisms has a chance to affect them before being bound by soil (Kremer et al. 2005; Johal and Huber 2009).

Recent work by Zobiole et al. (2010b), not cited by APHIS, provides the most detailed picture to date of changes in rhizosphere microorganisms in response to glyhposate application at different stages of soybean development for both first-generation Roundup Ready and second-generation Roundup Ready 2 Yield varieties. These studies show that Fusarium spp. increased, and rhizosphere fluorescent pseudomonads, Mn-reducing bacteria, and IAA [growth hormone]-producing rhizobacteria all decreased after glyphosate was applied, relative to the same cultivars grown without glyphosate. Root and shoot biomass also decreased. They conclude that "[g]lyphosate applied to GR soybeans, regardless of cultivar, negatively impacts the complex interactions of microbial groups, biochemical activity and root growth that can have subsequent detrimental effects on plant growth and productivity".

Although earlier work focused on the micronutrient manganese and under what conditions its levels were reduced by glyphosate (Zobiole et al. 2010c and references therein), more recent studies show that glyphosate can also lead to lower levels of other nutrients, such as nickel (Zobiole et al. 2010a) or iron. Nitrogen fixation and other aspects of nitrogen metabolism were inhibited by glyphosate in some situations (Zobiole et al. 2010a). Photosynthesis can also be affected (Zobiole et al. 2010a).

These effects of glyphosate on microorganisms depend on translocation and thus are likely to be more persistent in the rhizosphere of Roundup Ready crops that keep on ticking after applications, as opposed to non-Roundup Ready crops and weeds that are going to die. Nevertheless, many of the early studies of pathogen interactions with glyphosate predate Roundup Ready technology. The experiments led to an idea that glyphosate in field situations works synergistically with fungal pathogens to kill weeds (Levesque and Rahe 1992).

Gressel (2010) has renewed interest in this phenomenon, where in Table 3, he shows that glyphosate synergizes many mycoherbicidal pathogens, perhaps by binding cations needed for plant defense mechanisms. Since this would be independent of the metabolic mode of action of glyphosate, it should occur in Roundup Ready crops just as in conventional ones.

Additionally, scientists at Purdue are currently examining the role of glyphosate-pathogen synergy in weed resistance to glyphosate (Schafer et al. 2009):

> "Our findings confirm that the insensitive biotype of each weed was more sensitive to glyphosate in unsterile soil, than the sensitive biotype in sterile soil. Soil microbes play an important role in the mode of action of glyphosate. Thus, it is possible that the evolution of resistance to glyphosate may stem not only from the resistance to the herbicide itself, but also resistance to soil microbes. Further research will investigate whether or not the insensitive biotypes of common lambsquarters and giant ragweed studied here exhibit elevated levels of resistance to soil microbes."

Sugar beets are plagued by a number of fungal and bacterial pathogens, as discussed in the DEA. A study by Larson et al. (2006) with a different Roundup Ready sugar beet event concluded that glyphosate sometimes led to higher levels of disease severity, but was dependent on certain fungal isolates and other conditions. They did not conclude that the glyphosate effect was due to interaction of glyphosate with the fungal EPSPS and so assumed that increased disease severity was plant mediated. Larson, now working for Syngenta, has concluded that the increased disease severity with glyphosate observed in her greenhouse experiments may have been due to herbicide stress in general, and that the appropriate control should have been conventional herbicides so that all individuals would be stressed (Larson 2010, cited in DEA). However, Roundup Ready technology is supposed to allow glyphosate treatment without stressing the crop, so Larson's original experiments using "no herbicide" controls should have been appropriate.

Larson et al. argue they were not able to reproduce glyphosate-enhanced disease severity in the field with H7-1 sugar beets (Larson 2010, cited in DEA), although the results of the field tests are not published so cannot be fully examined to determine, for example, if the controls were grown with or without conventional herbicides.

Given the complexities of both soil microbial ecology and disease-plant interactions, the fact that glyphosate is erratic in enhancing diseases should not be surprising. What this means for diseases if the H7-1 system is adopted is unknown at this time.

If H7-1 sugar beets do behave similarly to Roundup Ready soybeans by changes in rhizosphere microorganism communities in response to glyphosate, then assuming the sugar beets themselves do not succumb to more severe disease symptoms, they could leave behind higher inocula of disease organism that could infect crops in rotation. Larson et al. (2006) alluded to this possibility in their discussion: "A future direction includes understanding the impact of GR crops on rotation. Increases in Rhizoctonia root rot could increase the soil pathogen population and affect other susceptible crops in rotation with GR sugar beet, such as dry bean, soybean and corn."

Neither Larson et al. nor APHIS cited the work of Fernandez on the association between previous glyphosate use and subsequent Fusarium Head Blight (FHB) in wheat and barley. Fernandez (2009) reviewed work in this field, much of it her own, and concluded for wheat that:

> "Previous glyphosate application, nested within tillage system, was the only agronomic factor significantly associated with higher FHB levels every year of the study (Tables 3 and 4). Glyphosate's effect on the FHB index was not influenced by environmental conditions as much as for other agronomic factors whose effects on disease levels were inconsistent from year to year. Under minimum-till, application of glyphosate at least once in the previous 18 months significantly increased the mean FHB index and the proportion of fields in the high FHB index class every year."

Fernandez further observed similar associations between previous glyhposate applications to fields then planted in barley and various Fusarium diseases.

> "These studies document the positive association of glyphosate with pathogenic Fusarium spp., including F. avenaceum, F. culmorum and F. graminearum, in spikes/kernels and subcrown internodes of wheat and/or barley, and in residues of these crops almost a year after harvest. The exact nature of these associations was not determined. Previous research has shown that herbicides, including glyphosate, can inhibit or stimulate the growth of fungal pathogens, and can either increase or decrease disease development through direct or indirect means (Altman, 1993; Levesque and Rahe, 1992). Levesque and Rahe (1992) showed evidence that herbicides can have a direct effect on various components of the soil microflora, such as plant pathogens, antagonists, or mycorrhizae, which can potentially increase or decrease the incidence of plant disease. Pathogens able to infect weeds can also increase their inoculum potential after weeds have been sprayed with herbicides, which could subsequently affect host crops."

Clearly, more field studies need to be done to discern the effects of increased glyphosate use on specific microorganisms, and their role in diseases on H7-1 sugar beets and rotation crops. The fact that increased incidence of disease was not evident in the first two years of H7-1 adoption or that yields were "good" does not constitute a careful study of the potential for glyphosate-facilitated changes in Fusarium populations, in particular. Such changes could linger in particular fields long after glyphosate use ended.

<u>Comments Concluded:</u>

APHIS minimizes the impacts of increased glyphosate use in H7-1 sugar beets by saying that "[g]lyphosate has been used with 95% of sugar beet production, but accounts for only 1% of total glyphosate used in US agriculture." (DEA p. 72, Affected Environment: Weed Management) However, in the sugar beet growing regions and on the specific acreage where they are grown, management practices for H7-1 sugar beets are intense and just as likely to impact human and animal health and the environment locally as practices in crops grow on a larger scale overall. APHIS has not used the latest scientific studies to carefully assess the consequences of increased glyphosate use from Alternatives 2 or 3, and thus has overstated the negative consequences of using less glyphosate under Alternative 1.

In light of the multiple shortcomings in the DEA, I recommend Alternative 1: no action pending approval of the forthcoming EIS.

Sincerely,

Martha L. Crouch
Science Consultant, Center for Food Safety

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