Independent scientific biosafety assessment of the application for commodity clearance of transgenic soybean, DAS-68416-4

William H.L. Stafford, PhD, and Gareth Jones

The application is for the commodity clearance of a new genetically modified soybean, DAS-68416-4, that is tolerant to two herbicides:- 2,4-dichlorophenoxyacetic acid (2,4-D) and glufosinate ammonium. Dow Agrosciences states that this new transgenic will provide substantial benefits to growers by limiting yield losses from weed pressure.

Rationale for this application

As stated by Dow, 'this commodity clearance application is to support the potential shortage of soybean in South Africa and the subsequent need to import grain from exporting countries that may be commercially growing DAS-68416-4 and other genetically modified soybean (p.87)'. This is mainly to feed South Africa's rapidly growing poultry industry. Despite a huge increase in domestically produced soybean in recent years (from 100,000ha cultivated in 1999 to 418,000ha in 2011), production costs (including feed) are squeezing the margins of many domestic producers. A recent, well publicised trade spat with Brazilian poultry producers highlights this (ACB, 2011). The underlying purpose is to open markets for a new generation of herbicide tolerant crops that have been developed in the United States in response to an epidemic of glyphosate tolerant weeds. This will be expanded upon below.

Lack of data

Of general concern is the data that is required for independent assessment (and should not be considered CBI) have not been included eg. [2009a-Attachment A [CBI-DELETED: Section 68(a),(b) and (c)ii of the Promotion of Access to Information Act] and many others in the dossier. It is increasingly difficult to independently assess an application when the scientific data is withheld.

Contents

Characterisation of the DAS-68416-4 transgenic

The DAS-68416-4 transgenic soybean was engineered by the introduction of the *aad-12* gene, from *Delftia acidovorans*, expressing the protein aryloxyalkanoate dioxygenase (AAD-12) for tolerance to 2,4 D and the *pat* gene from *Streptomyces viridochromogenes* for tolerance to glufosinate herbicide. The transgenes are driven by the constitutive promoters; CsVMV promoter from the cassava vein mosaic virus, (a plant pararetrovirus belonging to the caulimovirus subgroup and AtUbi10 polyubiquitin promoter from Arabidopsis thaliana (Verdaguer et al., 1996; Norris et al., 1993). A matrix attachment region (MAR) of RB7 from *Nicotiana tabacum* was included at the 5' end of the aad-12 gene to potentially facilitate expression of the aad-12.

Of immediate concern, characterisation studies of the AAD-12 protein rely upon a microbially-produced surrogate, using *Pseudomonas fluorescens (Pf)* (p.52). Not all proteins produced from the same gene are physically identical copies. Sometimes a small minority of copies can be physically and functionally distinct. Not all organisms produce the same variety of minority forms, either, and therefore the family of protein isoforms made in bacteria may not fully represent the family of isoforms made in the GMO. Because of this, studies based on surrogate sources of protein can rarely, if ever, substitute for those based on protein taken from the actual GMO, if the developer is attempting to make a credible claim that adequate hazard identification standards have been met (Freese, W. & Schubert, D., 2004). As part of the South African National Biodiversity Institute's (SANBI) MON810 study, proteins expressed in bacterial and maize hosts were found to differ in protein size. The author concluded that "differences in size and possible bioactivity between maize expressed and bacterially expressed CryIAb 'Bt' proteins suggest that the practice of using the bacterial version as a replacement for maize versions of the same transgenic protein in safety testing should be re-evaluated." *(Quist, 2011).*

Southern blot

There are several experiments with data presented using Southern blots to characterise the transgenes in terms of integrity of the introduced transgenes/ transgenic cassette. The data presented on Southern blotting was used to verify the integrity of the transgenes and that no other unwanted genetic elements from the plasmid vector have been introduced into the DAS-68416-4. For example, experiments were carried out to test whether the *strepR* gene (encoding Streptomycin resistance) was present in the DAS-68416-4 transgenic soybean. However, in these Southern blots there are considerable differences in band intensity and the author has noted that these are attributable to differences in amounts of DNA loaded into each well because of different recovery after digestion "(eg1 on page 23 The relatively strong signals in Lane 10 and 15 were due to a larger amount of DNA recovered after digestion). Conversely, on page 29, "Note: The relatively weak signal in Lane 7 was due to a lesser amount of DNA recovered after digestion". **This is a grave omission in the Southern blotting technique. It is essential to load the**

same amount of DNA (or more specifically, genome equivalents) in each lane so that inter-lane comparisons and conclusions as to whether a signal (and hence genomic sequence) is present or absent, can be made.

There are several ways to ensure that this is the case. Usually a restriction enzyme digests are set up with the same amount of DNA in each tube and, after completion, the DNA solution is loaded onto the well of an agarose gel for electrophoresis and Southern blotting. Sometimes there are inhibitors in the DNA that necessitates using larger reaction volumes in the restriction enzyme digests; in which case the DNA needs to be precipitated and re-suspended into a smaller volume. Obviously, losses in DNA can occur during this procedure so it is standard to carry out a quick spectroscopic determination of the DNA concentration (Absorbance at 260nm) and to adjust the volumes that are loaded into each lane of the gel to ensure that the same amounts are loaded. Clearly, this was not carried out! The conclusions that have been made from these Southern blots are therefore unsubstantiated, since, for example, the absence of a StrepR signal could be due too little DNA present in that lane to be detected.

To summarise, there have been several Southern blotting experiments to analyse the characteristics of the transgenic, but the lack of equal genome equivalents in different samples means that the conclusions and claims from the transgenic characterisation are unsubstantiated.

Other transgene characterisation techniques?

Aside from the problems with the Southern blotting method, **there have been no additional experiments to address unintended genomic effects. For example, there is no evidence presented on the *in vivo* effects of the transgenic construct (except for inheritance of the desired traits) with and without the MAR sequence.** Our understanding of the effects of the genome-wide effects of MAR (as well other elements of the transgenic cassette) is poorly understood and experiments to reduce the risks of unintended genetic effects (rearrangements, disruptions, deletions in the genome) and pleiotropic effects have not been addressed. The genome integrity and stability of the cassette cannot accurately be determined because of failure to properly address this issue using the limited method of Southern blotting. Techniques such as repPCR, RAPD and comparative genome hybridization (CGH) have been shown to be effective in establishing genome similarity (Bao et al. 1993, Pinkel and Albertson 2005). This is required since fragmenting and scattering of the transgenic cassette in the genome (transpositions with rearrangements and deletions) may result in loss of the primer binding sites or a large distance (>10kBp) between genetic elements of the cassette, giving in false negative results by when detection is carried out by standard Southern blotting.

There are also concerns with the use of poorly understood genetic elements such as the MAR sequence that is thought to facilitate attachment of the AAD to the matrix or scaffold of the nucleus. Since the nuclear matrix can be detected only under certain preparation conditions, the *in vivo* existence has been somewhat controversial. MARs have also been shown to increase expression of transgenes and to reduce the incidence of gene silencing (Han et al., 1997; Abranches et al., 2005; Verma et al., 2005). **A MAR was included at the 5' end of aad-12 PTU to potentially facilitate the expression of AAD-12 in transgenic plants, but the exact effects on expression, localisation of AAD-12 and other genome-wide effects have not been studied.** There are also biosafety concerns with viral promoters and synthetic genetic elements that are new to nature and have not been tested for biosafety in terms of genome-wide effects, genome stability and horizontal gene transfer. Viral promoters may increase rearrangements/ deletions and affect cassette integrity, genome stability and gene flow. This presents new biosafety risks, including:

- Increased recombination (rearrangements, deletions, insertions). There is evidence from the laboratory (Koholi *et al.* 1999) and field studies (Quist and Chapela 2001, Collonier *et al.,* Ho et al. 2000) that viral promoters are recombination 'hotspots'.
- The viral promoters result in very high expression levels that may result in unintended (pleiotropic) effects from the expressed transgenes.
- Viral promoter will have and increased recombination potential with other viral elements and the creation of new viruses (Wintermantel *et al.* 1996, Vaden and Melcher 1990, Greene *et al*. 1994).

Food and feed safety issues

Composition analyses of DAS-68416-4 soybean have shown that the contents of protein, fiber, carbohydrates, fat, ash, minerals, fatty acids, amino acids, vitamins, secondary metabolites and anti-nutrients are equivalent to that found in non-GM control soybean **with comparable genetic background**, **representative commercial lines**, and to the published range of values in the literature (Phillips and Lepping, 2010b-Attachment P). Of concern in the nutritional profiling is that the comparison of DAS-68416-4 should be with the wild type parental grown under the same conditions (the appropriate control), and *not* "representative commercial lines, and to the published range of values in the literature " (an inappropriate control that may hide unintended effects).

Toxicity assessment

'An acute oral toxicity study with AAD-12 protein was conducted in mice at a level of 2,000 mg AAD-12 / kg after adjustment for purity. All animals survived and no clinical signs were observed during study. All animals gained weight by study termination on day 15. Information on the size and composition (for example, were the mice infant or adult) of the study is lacking. Why was the study terminated after 15 days? Regulatory bodies such as the European Food Safety Authority (EFSA), for example, requires at least 28 days (EFSA, 2008).

As the genes used in present-day genetic engineering ensure that GM food is unlikely to be highly poisonous. "Toxicity" therefore is an unhelpful concept that does not easily lend itself GMO in animal nutrition: potential benefits and risks to quantitative assays. In contrast nutritional studies, in which GM crop-based diets are fed to young growing animals, should reveal their possible harmful effects on metabolism, organ development, immune/endocrine systems and gut flora, which together determine the safety of the GM crop and the development of the young into healthy adults (p. 521-522 Pusztai, A. & Bardocz, S., 2006). Stating that 'all animals survived and no clinical signs were observed during the study' (over 15 days) gives no indication of the longer term, chronic effects, which are far more relevant to a food product that could potentially be consumed over a much longer period than the study covers.

Nutritional assessment

The only experiments to assess the potential nutritional equivalency and toxicity of DAS-68416-4 soya is a bird feeding study and the feeding of bacterially expressed Pat and Aad-12 proteins in mice. The bird feeding trial was carried out to observe effects on mortality and weight gain after 41 days. The report states, "There were no adverse effects of the consumption of DAS-68416-4 soybean on mortality or morbidity, general clinical observations, body weight, body weight gain, or feed conversion". However, an important statistical analysis of chicken feeding studies conducted to 2004 found that most were incapable of detecting moderate to low level health effects in the short time of testing on chickens, and thus may miss important adverse effects that would be possible over the lifetime of humans (Roush, W.B. & Tozer, P.R., 2004). This re-analysis assessed the power of tests to determine the adequacy of the experimental design being used by developers in studies provided to decision-makers attesting to the wholesomeness and safety of GMOs as food. The authors found that the "results of the survey of the literature showed, in general, low power of statistical tests for feeding experiments involving non-GM grains or in those cases when GM and non-GM grains were compared in poultry feeding experiments. These results suggest that care needs to be taken when designing experiments for bioequivalence of grains fed to poultry" (p. E110 Roush, W.B. & Tozer, P.R., 2004).

Unfortunately, such was the paucity of information supplied by the applicant that it is nigh on impossible to assess the robustness of the said feeding trial. For example, how large was the study group, and were any birds withdrawn / changed during the feeding trial? No information is given about the control group, nor the control groups diet, beyond being 'non-modified **near isogenic, or standard commercially available soybean.** To reiterate the point raised above, the control diet should be the conventional comparator of the GMO which was produced simultaneously and under identical conditions (e.g., grown side by side if a GM plant). There should be a second control diet with the conventional comparator "spiked" with the compounds known to be different between the GMO and the comparator and isolated from the GMO. This diet controls for non-specific effects due to the process of creating the GMO (Pusztai, A. & Bardocz, S., 2006).

It is stated (p.78) that "*Daily feed intake was 3.7% less for male birds fed diets containing the transgenic soybean meal compared with those fed the non-transgenic near-isogenic soybean meal*…" (Italics added for emphasis) Was this daily feeding intake not significant?

Most food safety experts are in agreement that severe and acute effects from food will be rare (Schilter, B. & Constable, A., 2002). Therefore, GM food safety testing should be designed to test for sub-chronic effects that might produce mild or ambiguous symptoms. It is common for too few replicates to be included in developers' feeding studies (Gallagher, L.M., 2007; Roush, W.B. & Tozer, P.R., 2004; Seralini, G-E. *et al.*, 2007). Finally, it is not clear from the information presented, if the DAS-68416-4 used in the feeding study contained residues of 2,4-D and glufosinate-ammonium, the two herbicides that will be applied directly to the crop.

Potential risks to human and animal health

GM feed has been found to affect the health of animals that eat it. For example, GM DNA from soy was detected in the blood, organs and milk of goats. An enzyme, lactic dehydrogenase, was found at significantly raised levels in the heart, muscle and kidneys of young goats fed GM soy. This enzyme leaks from damaged cells during immune reactions or injury, therefore high levels may indicate problems.[1] GM DNA in feed has been taken up by the animal's organs and detected in sheep,[2] pigs[3,4], and rainbow trout.[5]

It is, therefore, a gross omission in the consideration of 2,4,D herbicide residues that will be ingested by humans and animals from soya food and feed. In the literature, experiments in which lactating rats were fed low doses of 2,4-D revealed that the chemical inhibits breast feeding from mother to pup and as a consequence, led to weight loss in the offspring. 2,4-D and its formulations have been found to cause chromosome and DNA damage in hamster ovary cells,

the bone marrow and developing sperm cells of mice, and sister chromatid exchange (which has been linked to the formation of tumours) in chicken embryos (Gonzalez 2005, 2001, Madrigal-Budhaidar 2001, Arias 2003). Given the evidence of 2,4 D causing cancers in humans, particularly non-Hodgkin's lymphoma, it is perhaps unsurprising that Sweden, Norway and Denmark have banned 2,4-D (Boyd 2006).

Environmental, animal and human health issues from 2,4-D and Glufosinate

2,4-D

There will also be environmental, animal and human health problems from the fact that 2,4-D is a volatile herbicide that is prone to drift beyond the field of application and is a probable carcinogen. Particularly sensitive crops include grapes tomatoes, cotton, soybeans, sunflower, and lettuce (Walker 2011). Two surveys of USA pesticide regulators establish that 2,4-D drift is already responsible for more episodes of crop injury than any other pesticide (AAPÇO 1999, 2005). Introduction of 2,4-D crops will greatly increase drift injury to crops by increasing the magnitude and duration of 2,4-D application. Although Dow claims to have a less drift-prone formulation of 2,4-D, its efficacy has not been independently validated. Conventional farmers are likely to lose crops while organic farmers could lose both crops and certification.

2,4-D and the closely related 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) formed the Agent Orange defoliant used in the Vietnam War. The World Health Organization's International Agency for Research on Cancer classifies chlorophenoxy herbicides, the predominant member of which is 2,4-D, as "possibly carcinogenic to humans" (IARC 1987) for multiple types of cancer. One possible cause of 2,4-D's toxicity is the continuing presence of dioxins, a difficult and perhaps impossible to eliminate contaminant of the production process. The latest available industry tests on 2,4-D continue to find its presence (EPA Dioxins in 2,4-D). Numerous epidemiological studies have reported an association between exposure to 2,4-D and non-Hodgkin's lymphoma (a cancer of the white blood cells that kills 30% of those afflicted) and also an association with soft-tissue sarcoma (Chiu 1992, Hardell et al. 1979, 1981, 1999, 2001, McDuffie 2001) . Additional evidence of a link between 2,4-D and non-Hodgkin's lymphoma comes from studies of canine malignant lymphoma conducted by National Cancer Institute scientists, which showed increased incidence of the canine equivalent of NHL in dogs exposed to 2,4-D-treated lawns (Hayes et al 1991, 1995).

Glufosinate[6]

Cardiovascular effects

Two dogs fed 8mg/kg bw/d for 10 and 14 days died of heart and circulatory system failure. Basta, a formulation of glufosinate, caused decreased blood pressure and increased heart rate at low doses, and decreased heart rate in very high doses, in rats.

Nervous system

Glufosinate-ammonium is structurally similar to glutamate, an amino acid important to the proper functioning of the nervous system, and the herbicide causes signs of neurotoxicity in most species of animals for which it has been tested. Glutamate is an important neurotransmitter, especially in brain function, and the herbicide causes activation of the neurotransmitter receptors for glutamate (Matsumara et al 2001). It also stimulates production of nitric acid in the brain, through stimulation of Nmethyl D-aspartate receptors. The increased production of nitric acid is held to be responsible for the epileptic (tonic-clonic) seizures resulting from exposure to glufosinate-ammonium. Developing brains appear to be particularly susceptible to glufosinate-ammonium. Exposure of the foetal brain of rats (through maternal exposure) resulted in functional abnormalities in the offspring.

Reproductive & developmental effects

Following studies on rats, mice and rabbits, the Swedish National Chemicals Inspectorate (KEMI) has concluded that glufosinate-ammonium is toxic to reproduction and the product should carry risk warnings.

Residues in Food

The European Food Safety Authority (EFSA) found that residues of glufosinate in potatoes to be as high as 0.5mg / kg, and that these would not be altered by cooking in water. EFSA concluded that these residues pose an acute risk for small children, as they were 114% of the acute reference dose. Residues of glufosinate remain stable in frozen GM maize for up to 2 years. Where glufosinate is applied to GM glufosinate tolerant crops, it degrades into the metabolite N-acetyl-glufosinate (NAG). According to Jewell & Buffin (2001), data from Aventis indicates that NAG can be reconverted into the active herbicidal form by micro-organisms in the digestive tract of warm-blooded animals, including humans.

Environmental risks

KEMI has noted that the use of glufosinate poses an unacceptable risk to beneficial insects, and when used in conjunction with GM crops, could pose a long term risk to bird populations. Studies from 12 agricultural and 10 forest soils in Canada found that glufosinate selectively reduced the number of fungi and bacteria in soils. In agricultural soils it reduced fungi by 40% and bacteria by 20%. IT also significantly inhibits decomposition of cellulose in soils and is toxic to nitrogen-fixing soil bacteria.

Gene escape and unknown environmental effects

In the application it is stated:

"Detail whether the genetically engineered plant is able to initiate resistance, in any biotic component of the environment, to any biologically active foreign gene product.

As this is an application for commodity clearance of DAS-68416-4, i.e. use as food, feed or for processing, and not an application for release of DAS-68416-4 into the environment of South Africa, this question is not applicable."

The monitoring of unintended effects in terms of gene escape should also include accidental escape of seeds imported for commodity clearance that may be planted and propagated, as well as horizontal gene transfer (HGT) from soya and soya products to intestinal bacteria and to soil bacteria. These HGT can occur when soya feed containing transgenic DNA may transfer to intestinal bacteria during feeding and/or pass undigested through the ruminant tract whereby the animal manure remains in contact with soil bacteria, until complete decomposition. Evidence to date shows that both these pathways for gene escape into the environment are possible and likely:

(i) Current evidence shows that horizontal gene transfer (HGT) to bacteria does occur, is significant and occurs at a high frequency when sequence homology is present. Horizontal transfer of DNA occurs at very low frequency under laboratory conditions, for example, Acinetobacter, a soil- and water-borne bacterium (Gebhard and Smalla, 1998) or Streptococcus gordonii, a cause of dental cavities and heart valve infection (Mercer et al., 1999. Crucial to the detection of HGT is the use of assay systems that are sensitive enough to detect even very rare events. The detection limits of some culture-based methods (typical detection limit of 10^{-8}–10^{-11} HGT events per bacterium) can exceed expected rates of HGT (10^{-16}–10^{-17}) by several orders of magnitude. Harbouring a plasmid with an *nptII* gene containing a small deletion (hence nonfunctional) was used to detect the frequency of HGT from plants containing transgenic DNA. The *nptII* gene transforms naturally competent cells of the soil bacteria Pseudomonas stutzeri and Acinetobacter BD413 with the same high efficiency as nptII genes on plasmid DNA ($3x10^{-5}$ - $1x10^{-4}$) despite the presence of a more than 10^{6} fold excess of plant DNA. However, in the absence of homologous sequences in the recipient cells the transformation dropped by at least about 10^{8} fold - 10^{9} fold. This indicates that recombination in bacteria is most efficient where sequence homology is present (de Vries J, Wackernagel W 1998). The *aad* and *pat* genes all have many gene homologs in soil bacteria indicating an increased risk for horizontal gene transfer. Furthermore, a study carried out by the British Food Standards Agency, to determine if transgenic

DNA transferred to bacteria of the human gut by HGT, found that this did indeed occur (Netherwood 1990).

(ii) After almost three decades of world-wide use, there is confirmed herbicide resistance to glyphosate exists in *Lolium rigidum* (annual ryegrass) in Australia and South Africa; *Lolium multiflorum* (Italian ryegrass) in Chile, *Eleusine ind*ica (goosegrass) in Malaysia; and *Conyza canadensis* (marestail) in the eastern parts of the United States of America.

There may other consequences of gene escape and hybridisation including the spread of herbicide tolerance, non-target effects on other plants, animals (Cui and Xia 1999, Hillbeck 1999) and soil microorganisms important for soil function such as nutrient availability and cycling (Benbrook 1999 and Kowalchuk 2003).

This new genetic material acquired by HGT will only be retained if it has a selective advantage. The regular and wide-spread application of glufosinate and 2,4 D herbicides will ensure a selective advantage. Several "herbicides" are in fact antibiotics produced by Actinomycete bacteria to kill competing bacteria in the soil. Therefore acquiring glyphosate and glufosinate resistance genes may acquire a selective advantage *per se* to soil microbes and change the soil microbe structure and function. Selective pressure for retaining the resistance gene will include application of herbicide, but a stress response facilitates the horizontal gene transfer and spread of resistance genes so that several other stresses such as soil tilling could increase dissemination of mobile genetic elements carrying resistance genes. For example the SOS response—induction of specific genes in response to DNA damage—alleviates the repression of genes necessary for the horizontal gene transfer of the mobile genetic element conferring resistance to the antibiotics chloramphenicol, trimethoprim, streptomycin, and methoxazole. (Beaber *et al*., 2003). Mobile genetic elements have played a key role in spreading resistant genes amongst bacterial populations and contribute to multiple resistance of bacterial pathogens (Slayers and Shoemaker, 1994; and Witte, 1997). **Therefore, there are risks associated with the spread of resistance genes amongst soil bacteria, even when there is no selection for the transgenic construct *per se.* The effects from these changes in soil biodiversity and soil ecosystem functioning have not been considered.**

There is also the possibility that human error will result in the inadvertent mixing, spread and planting of DAS-68416-4 seeds (and DNA from processed soy). This includes intermingling of GM and non-GM seeds in shipments. Monitoring of and reporting of such events on the Biosafety Clearing House (BCH) is haphazard, to say the least. Legally permitted 'adventitious presence' of GM seed in non-GM shipment (up to 0.9% in the EU for example) is another avenue through which GM seed can pass undetected.

Therefore, DAS-68416-4 seeds may co-mingle with non-GMO soya and be propagated in South Africa without being detected (only when soya is exported would the soya seeds require testing as per the Biosafety Protocol). The environmental monitoring for gene escape should therefore include using appropriate monitoring programmes that use molecular methods to track gene escape (ie. Gene specific PCR, DGGE microbial profiling, microarrays; see Kowalchuk *et al.* 2003). It would also be sensible to limit the commodity clearance to milled (or further processed) soya to avoid the import of viable soya seeds into South Africa which would reduce these risks.

Re-exports to other countries?

Finally, there is a risk that should this new variety be permitted for import into South Africa, it could be re-exported in other shipments of GM soya. According to Dow, 'exports of soybeans from South Africa over the last few years have been very insignificant and it is therefore unlikely that the grain imported into South Africa would be exported. However, the South African Grain Information Service (SAGIS) states that soybean exports for 2009 and 2010 were 155,600 and 121,300 tons respectively. In 2012 the Executive Council (EC) has granted export permits for GM soy with a combined volume of nearly 300,000 tons.[7]

Increasing Resistance with increased pesticide use

In the application it is stated: 5.2 Detail what methods are available to minimise the risk of resistance developing in the environment.
Considering that this is not an application for environmental release, this question is not applicable.

Aside from the above-mentioned biosafety risks of gene escape from soya imported for commodity clearance (food and feed); this application is likely, if approved, be followed by an application for cultivation and general release. This will increase the use of herbicides and accelerate the development of resistance. Herbicide-tolerant (HT) crop systems such as DAS-68416-4 involve post-emergence application of herbicides to a crop that has been bred or genetically engineered to survive application of the herbicide(s). These HT crop systems promote more rapid evolution of herbicide-resistant weeds, compared to non-HT crop uses of the associated herbicides, because of more *frequent* and *extensive* use, leading to *over-reliance* on these pesticides.

Glyphosate herbicide history reveals the pattern of resistance to be expected with glufosinate and 2,4D. Glyphosate was first introduced in 1974, but despite considerable use of the herbicide for two decades, only a few isolated populations of resistant weeds emerged as a result of intensive glyphosate use in orchards (e.g. Malaysia, Chile, California) or in wheat production

(Australia). However, since 1996 there has been a rapid introduction of transgenic crops resistant to glyphosate:- introduction of Roundup Ready (RR) crop system- RR soybeans, followed by RR cotton, RR canola and RR maize. For example, by 2011 over 136 million ha of GM crops (representing 85% of all GM crops) planted worldwide were herbicide tolerant.[8] Far from leading to a reduction in overall pesticide use, as promised by the biotechnology industry, between 1996 and 2009 HT crops resulted in the use of an additional 173,000 tons of herbicides in the United States.[9]

According to the International Survey of Herbicide-tolerant Weeds (ISHRW), multiple populations of 23 weed species are glyphosate resistant in one or more countries today. Glyphosate-resistant weeds have emerged overwhelmingly in soybeans, cotton and maize in countries, primarily the U.S., where RR crop systems predominate. The first glyphosate-tolerant weed population confirmed in the U.S., reported in 1998, was rigid ryegrass, infesting several thousand acres in California almond orchards. By 2012, as many as 239,851 sites on up to 16,683,100 acres were documented to be infested by glyphosate-tolerant weeds (CFS GR Weed List 2012): this represents over 70-fold increase in number of sites and 7-fold increase in area.

Industry's 'solution' to glyphosate tolerance

In the United States the situation is approaching breaking point for many farmers. Executives at DuPont estimate that by the middle of this decade glyphosate resistant weeds could affect up to 40% of US farmland. Consequently, the biotechnology and seed companies have channelled hundreds of millions of dollars into developing new GM crops resistant to numerous highly toxic chemical herbicides, including 2,4-D, glufosinate, and diacamba (Kilman, 2010). Of the 20 GM events currently pending approval in the USA, 14 are new herbicide tolerant varieties, 6 of which for alternatives to glyphosate (USDA, 2012).

Crop	Company	Trait	Event
Cotton	Bayer	Glufosinate	T303-3
Maize	Pioneer	Glufosinate	DP-ØØ4114-3
Soybean	Monsanto	Dicamba	MON-877Ø8-9
Soybean	Dow	Glyphosate and Glufosinate	DAS-444Ø6-6
Soybean	Bayer	Glyphosate and Isoxaflutole	FG72
Soybean	BASF	Imidazolinone	BPS-CV127-9

Source: USDA-APHIS

2,4-D is the most important and widely used member of the synthetic auxin herbicides and there are already 43 biotypes of 29 different weed species with resistance to synthetic auxin herbicides (ISHRW SynAux Weeds 4/22/12) with 16 weed species resistant 2,4-D (Mortensen et al (2012). Experts in the US have stated that 2,4-D tolerant crops will likely increase the overall use of 2,4-D in US agriculture from 12,000 tons to over 45,000 tons per year.[10] Therefore, DAS-68416-4 will

very likely foster rapid evolution of weeds resistant to 2,4-D and glufosinate and thereby contribute to multiple herbicide resistance. 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. This acceleration of emerging resistance can present a huge financial burden to tackle weeds that may require more toxic herbicides or manual control. It also presents a "tragedy of the commons", whereby weed susceptibility to glyphosate is the common resource being squandered. Further, there may be issues with liability since farmers who employ sound practices to prevent emergence of herbicide-tolerant weeds themselves can have their fields infested with resistant weeds from neighbours who foster resistant weeds by relying too heavily on herbicides and herbicide-resistance crop systems.

This situation is likely to be echoed on South Africa. Since 2010 field trials have taken place for 12 GM crop varieties tolerant to glufosinate, or combinations of glufosinate and glyphosate (see table below). Whether these new herbicide tolerant varieties are being imported, or cultivated within South Africa, the health implications are extremely disconcerting. Moreover, South Africa appears ill equipped to test for and monitor these GM crops, and their derived products, for pesticide residues.

Crop	**Company**	**Trait**	**Event**	**Year of last trial**
Maize	Pioneer	Glufosinate, glyphosate	TC1507 x MIR162 x NK603	2010
Maize	Pioneer	Glufosinate, glyphosate	TC1507 x MON810 x NK603	2011
Maize	Pioneer	Glufosinate	TC 1507 x MIR 162	2010
Maize	Pioneer	Glufosinate	TC 1507 x MON 810	2011
Maize	Pioneer	Glufosinate	TC1507	2011*
Maize	Pioneer	Glufosinate, glyphosate	TC 1507 x 59122 x NK603	2011
Maize	Pioneer	Glufosinate, glyphosate	TC 1507 x 59122 x MON810 x NK603	2011
Maize	Pioneer	Glufosinate, glyphosate	TC 1507 x NK603	2011
Maize	Pioneer	Glufosinate	TC 1507 x 59122	2011
Cotton	Bayer	Glufosinate	BGII x LL cotton 25	2011
Cotton	Bayer	Glufosinate, glyphosate	GlyTol x LL Cotton 25	2010
Cotton	Bayer	Glufosinate	BG II x GlyTol x LL Cotton 25	2011

Source: ACB (2012).

Lack of pesticide residue monitoring

If the use of 2,4-D and glufosinate increases in line with the introduction of GM crops tolerant to them, as has been the case with glyphosate, higher residues of these pesticides in our food supply is a genuine possibility. Consequently, the issue of pesticide residues testing and monitoring will become of paramount importance. However, leading health professionals in South Africa have expressed concern that there is virtually no local laboratory capacity to test for 2,4-D residues.[11]

This is consistent with research carried out by the ACB earlier this year into the state of glyphosate residue testing in South Africa's food system. In summary, we could find no laboratory with the capacity to test for glyphosate residues in food, while provincial and municipal environmental health inspectors, responsible for testing of imported and locally produced food, are hopelessly over stretched. According to information from the Health Professionals Council of SA (HPCSA), as of the 31st of March 2012, there were over 165,000 registered qualified health practitioners in South Africa. Of these 3,264 are classified under environmental health, with just 11 (eleven) 'food inspectors' among them. By way of comparison, there are 2,397 registered dieticians on the list.[12]

Lack of precaution

The GMO regulations prohibit the undertaking of an activity involving GMOs unless a 'suitable and sufficient assessment of the potential adverse effects to the environment, human and animal health and safety has been made'. Risk assessment is to be carried out on a case-by-case basis. The Regulations, paraphrasing the Precautionary Approach set out in the 1992 Rio Declaration on Environment and Developent, also stipulate that a lack of scientific knowledge or scientific consensus shall not be interpreted as indicating a particular level of risk, an acceptable risk or an absence of risk. This wording is very similar to the formulation of the Precautionary Principle used in Annex III of the Cartagena Protocol on Biosafety, to which South Africa is a party. The Precautionary Principle is also found in the National Environmental Management Act (Act 107 of 1998), which provides that 'a risk-averse and cautious approach is applied, which takes into account limits of current knowledge about the consequences of decisions and actions'.[13]

DAS-68416-4 has not been commercially cultivated anywhere in the world as yet, and thus has not been consumed by humans or animals either. Further, along with GM maize event DAS-40278-9, DAS-68416-4 will be the first GM event specifically engineered to be used in conjunction with 2,4-D herbicides. It is highly likely therefore that any imports of commodities derived from this event will contain high levels of pesticide residues. It is hard to understand how anyone could assert with any confidence that increased application of 2,4D is not related to a likely increased

presence of dioxin in environmental and mammalian systems if we are unable to monitor for dioxin.

Conclusion

It is our opinion that Dow's application for commodity clearance of DAS-68416-4 is wholly insufficient, and therefore without merit. It has been impossible to independently verify the claims for safety and efficacy made in the application, as most of the information vital to this has been omitted as 'confidential business information'. For example, the dossier makes reference to and draws conclusions about safety from animal feeding studies. Detailed information about the composition of the animal study group, comparator groups, and the diets of both groups has not been included. The molecular characterisation is inconclusive, relying solely as it does on Southern Blot analysis, with no additional experiments carried out to address further unintended effects. Another noticeable shortcoming is the use of surrogate proteins produced in a bacterial rather than plant host. Previous studies have indicated that expression of the same protein can vary depending upon the host it is produced in.

This GM soybean variety has also been engineered to tolerate applications of two chemical herbicides; 2,4-D and glufosinate ammonium. 2,4-D has been classified as 'possibly carcinogenic' by the World Health Organiation, and the results of numerous independent studies verify this. Glufosinate has been found to impact the cardiovascular, nervous and reproductive systems in mammals and rodents. The cultivation of this variety is likely to lead to huge increases in the use of both of these toxic herbicides in food production.

Although the current application is for commodity clearance (import for food and feed); this will undoubtedly be followed by an application for planting and general release. In either case, there is a lack of a proper monitoring program to track gene flow (by horizontal gene transfer, hybridisation, outcrossing) and ensure that these transgenes do not escape beyond their designated hosts. There are substantial new biosafety risks posed by the stacking of two herbicide resistant genes and releasing the transgenic into the environment; including the rapid evolution of herbicide resistance, the generation of herbicide resistant weeds and the increased use and exposure to toxic herbicides. The applicant also claims that there is minimal risk of DAS-68416-4 being re-exported, as South African soybean exports have been 'very insignificant' in the last few years. This is an extremely misleading statement; this year alone the Executive Council has granted export permits for GM soybeans with a combined volume of 300,000 tons.

Finally, it is highly debatable whether South Africa's food safety system has sufficient capacity to monitor imports of DAS-68416-4 for residues of both 2,4-D, glufosinate and their respective breakdown products. This is a new variety that, at the time of writing, has still not been commercially cultivated anywhere in the world. This alone merits a cautious approach in keeping with the Precautionary Principle. This is not only one of the key principles of the Cartagena

Protocol on Biosafety, to which South Africa is a party, but is also found in our own National Environmental Management Act. In light of this, and all of the evidence presented above, we respectfully request that the Executive Council should reject this commodity clearance application out of hand.

References

ACB (2012). **Hazardous Harvest: Genetically modified crops in South Africa, 2008-2012.**

ACB (2011). **Corporate concentration and control in the grain and oilseed value chain: A case study of the Bunge – Senwes joint venture**.

Arias E (2003). **Sister chromatid exchange induction by the herbicide 2,4-dichlorophenoxyacetic acid in chick embryos**. Ecotoxicol Environ Saf 55(3):338-43 2012. http://www.leg.bc.ca/cmt/39thparl/session-4/cp/reports/PDF/Rpt-CP-39-4-Report-2012-MAY-17.pdf

Bao, P.H.; Castiglione, S.; Giordani, C.; Li, W.; Wang, G.; Datta, S.K.; Datta, K.; Potrykus, I.; Sala, F. (1993). **State of the foreign gene and of the genome in transgenic rice *(*Orvza sativa).** Cytotechnology 11: S123125,

Benbrook, C.M. 1999. **Impacts on soil microbial communities needs further study**. AgBioTech InfoNet. June 24. http://www.biotechinfo.net/microbial_communities2.html Venkateswerlu G. and

Bennett, D (2006). "**2,4-D herbicide drift damage stuns east Arkansas cotton**," Delta Farm Press, 8/11/06. http://deltafarmpress.com/24-d-herbicide-drift-damage-stuns-east-arkansas-cotton

Bortolozzi AA, Evangelista DeDuffard AM, Duffard RO, Antonelli MC (2004). **Effects of 2,4-dichlorophenoxyacetic acid exposure on dopamine D2-like receptors in rat brain**. Neurotoxicol Teratol 26(4):599-605

Boyd, DR (2006). "**The food we eat: an international comparison of pesticide regulations,"** David Suzuki Foundation, 2006. http://www.davidsuzuki.org/publications/downloads/2006/DSF-HEHC-Food1.pdf

Bradshaw, L. et al (1997). "**Perspectives on glyphosate resistance**," Weed Technology 11:

Centre for Food Safety (2012). **Comments to EPA on notice of receipt of applications to register new uses of 2,4-D on enlist AAD-1 Corn and Soybean.**

CFS GR Weed Charts (2012). **Center for Food Safety's charts of glyphosate-resistant weeds by time of emergence, crop setting, and region, based on data from CFS GR Weed List (2012).**

Chiu BC, Blair A. **Pesticides, chromosomal aberrations, and non-Hodgkin's lymphoma**. J Agromedicine

Collonier C, Berthier G, Boyer F, Duplan M-N, Fernandez S, Kebdani N, Kobilinsky A, Romanuk M, Bertheau Y. **Characterization of commercial GMO inserts: a source of useful material to study genome fluidity.** Poster courtesy of Pr. Gilles-Eric Seralini, Président du Conseil Scientifique du CRII-GEN, www.crii-gen.org

de Vries J, Wackernagel W (1998). **Detection of nptII (kanamycin resistance) genes in genomes of transgenic plants by marker-rescue transformation**. Molecular and General Genetics 257:606-613

EFSA (2008). **Draft updated guidance document for the risk assessment of genetically modified plants and derived food and feed.** *EFSA J.* **727**, 1-135.

Egan, JF et al (2011). **"2,4-Dichlorophenoxyacetic aci (2,4-D)-resistant crops and the potential for evolution of 2,4-D-resistant weeds,"** PNAS 108(11): E37.

Freese, W. & Schubert, D. (2004). **Safety testing and regulation of genetically engineered foods.** *Biotechnol. Genet. Eng. Rev.* **21**, 299-324.

Gallagher, L.M. (2007). **Statistical and toxicological evaluation of two analyses on 90-day rat feeding study for MON863 transgenic corn.** *Environmental Science and Research, Ltd..* *www.nzfsa.govt.nz/consumers/gm-ge/r-gm-maize1.pdf* .

Gendel S. (1998). **The use of amino acid sequence alignments to assess potential allergenicity of proteins used**. Advances in Food and Nutrition Research 42, pp. 45-6

Gonzalez M, Soloneski S, Reigosa MA, Larramendy ML (2005**). Genotoxicity of the herbicide 2,4-dichlorophenoxyacetic and a commercial formulation, 2,4-dichlorophenoxyacetic acid dimethylamine salt.**

Greene, A.E. and Allison, R.F. (1994). **Recombination between viral RNA and transgenic plant transcripts**. Science 263, 1423-5.
Hagar, A. (2004). "Musings About Postemergence Herbicide Programs," IPM Bulletin, No.

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

Hardell L, Eriksson M**. A case-control study of non-Hodgkin lymphoma and exposure to pesticides**. Cancer 85:1353-1360, 1999.

Hartzler, B et al (2004). **"Preserving the value of glyphosate,"** article by 12 leading weed scientists, Iowa State University, Feb. 20, 2004. http://www.weeds.iastate.edu/mgmt/2004/preserving.shtml

Hebert VR, 2004. **Regional off-target movement of auxin-type herbicides**. Proceedings of the International Conference on Pesticide Application for Drift Management, Kona, Hawaii.

Hoar SK, Blair A, Holmes FF, Boysen CD, Robel RJ, Hoover R, Fraumeni JF. **Agricultural herbicide use and risk of lymphoma and soft-tissue sarcoma.** JAMA 256:1141-1147, 1986.

Huff, EA (2011). "**Court rules organic farmers can sue conventional, GMO farmers whose pesticides 'trespass' and contaminate their fields,"** NaturalNews, 8/3/11

IARC (1987). **IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Supplement 7**. International Agency for Research on Cancer, World Health Organization.

ISHRW SynAux Weeds 4/22/12. **List of weeds resistant to synthetic auxins,** downloaded

Kilman, S. (2010). **"Superweed outbreak triggers arms race,"** Wall Street Journal, June 4, 2010.

Knake, EL (undated). "**Phenoxy herbicide use in field corn, soybean, sorghum, and peanut production in the United States."**

Kohli A., Griffiths S, Palacios N, Twyman R, Vain P, Laurie D and Christou P (1999). **Molecular characterization of transforming plasmid rearrangements in transgenic rice reveals a recombination hot spot in the CaMV 35S promoter and confirms the predominance of microhomology mediated recombination.** Plant.J. 17,591-601

Kowalchuk G.A., Bruinsma, M., and van Veen, J.A. (2003) **Assessing responses of soil microorganisms to GM plants.** Trends in Ecology and Evolution Vol.18 No.8 .

Kruger, G.R. et al (2010a). **"Growth and Seed Production of Horseweed (Conyza canadensis) Populations after Exposure to Postemergence 2,4-D,"** Weed Science 58: 413-

Madrigal-Budhaidar E, et al (2001). **Induction of sister chromatid exchanges by 2,4-dichlorophenoxyacetic acid in somatic and germ cells of mice exposed in vivo**. Food Chem Toxicol 39(9):941-6

Marvier, M. 2001. **Ecology of transgenic crops.** *American Scientist* 89: 160-167. United States Environmental Protection Agency. 2001a.

McDuffic HH, Pahwa P, McLaughlin JR, Spinelli JJ, Fincham S, Dosman JA, Robson D, Skinnider LF, Choi NW. **Non-Hodgkin's lymphoma and specific pesticide exposures in men: Cross-Canada study of pesticides and health.** Cancer Epidemiol Biomarkers Prev. 10(11):1155-63, 2001.

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

Mortensen, DA et al (2012**). "Navigating a critical juncture for sustainable weed management,"** BioScience 62(1): 75-84.

Netherwood T, Martín -Orúe SM, O'Donnell AG, Gockling S, Graham J, Mathers JC, Gilbert HJ (2004). **Assessing the survival of transgenic plant DNA in the human gastrointestinal tract. Nature Biotechnology.** 22:204-209.

Pinkel D, and Albertson D.G. (2005). **Comparative genome hybridization.** Annual Review of Genomics and Human Genetics, Vol. 6, Pages 331-354

Pocock, J. (2012). **"Weed revolt marches on: glyphosate-resistant weed population grows rapidly,"** Corn and Soybean Digest, 1/17/12. http://cornandsoybeandigest.com/crop-chemicals/weed-revolt-marches-glyphosate-resistant-weed-population-grows-rapidly

Pusztai, A. & Bardocz, S. (2006). **GMO in animal nutrition: potential benefits and risks.** *Biology of Nutrition in Growing Animals* , 513-540.

Quist, D (2011). **Study to characterize the protein structure of the CryIAb transgenic (Bt) protein from MON810 maize and a comparison to the same protein produced in bacteria.** Monitoring the Environmental Impacts of GM maize in South Africa: The Outcomes of the South Africa-Norway Biosafety Co-operation Project (2008-2010).

Roush, W.B. & Tozer, P.R. (2004). **The power of tests for bioequivalence in feed experiments with poulty**. *J. Anim. Sci.* **82**, E110-E118.

Ryan, MR et al (2009). **"Weed-crop competition relationships differ between organic and conventional cropping systems,"** Weed Research 49: 572-80.

Seralini, G-E., Cellier, D. & Spiroux de Vendomois, J. (2007). **New analysis of a rat feeding study with a genetically modified maize reveals signs of hepatorenal toxicity**. *Arch. Environ. Contam. Toxicol.* DOI: 10.1007/s00244-006-0149-5, 596-602.

Sturtz N, Deis RP, Jahn GA, Duffard R, Evangelista de Duffard AM (2008). **Effect of 2,4-dichlorophenoxyacetic acid on rat maternal behaviour.** Toxicology 247(2-3): 73-79

Sturtz N, Jahn GA, Deis RP, Rettori V, Duffard RO, Evangelista de Duffard AM (2010**). Effect of 2,4-dichlorophenoxyacetic acid on milk transfer to the litter and prolactin release in lactating rats.** Toxicology Suppl):5485s-5488s, 1992.

USDA APHIS (2012). **Petitions for Nonregulated Status Granted or Pending by APHIS as of August 17, 2012**

USDA ERS AREI (2000). **Agricultural Resources and Environmental Indicators,** USDA Economic Research Service, Chapter 4.3, Pesticides, p. 5.

Vaden V.S. and Melcher, U. (1990). **Recombination sites in cauliflower mosaic virus DNAs: implications for mechanisms of recombination.** Virology 177, 717-26

Věra Bubeníková-Valešováa, Vladimir J. Balcarb, , , Hana Tejkalováa, Miloš Langmeierc and František Šťastnýa, d Neurochemistry International. (2006). **Neonatal administration of N-acetyl-l-aspartyl-l-glutamate induces early neurodegeneration in hippocampus and alters behaviour in young adult rats** Volume 48, Issues 6-7, May-June 2006, Pages 515-522

Wintermantel, W.M. and Schoelz, J.E. (1996**). Isolation of recombinant viruses between cauliflower mosaic virus and a viral gene in transgenic plants under conditions of moderate selection pressure**. Virology 223, 156-64.

[1] Tudisco et al. (2010). **Fate of transgenic DNA and evaluation of metabolic effects in goats fed genetically modified soybean and in their offsprings.** doi:10.1017/S1751731110000728

[2] Sharma R, Damgaard D, Alexander TW, et al (2006). **Detection of transgenic and endogenous plant DNA in digesta and tissues of sheep and pigs fed Roundup Ready canola meal**. *J Agric Food Chem; 54(5): 1699–1709.*

[3] Mazza R, Soave M, Morlacchini M, Piva G, Marocco A (2005)**. Assessing the transfer of genetically modified DNA from feed to animal tissues.** *Transgenic Res. Oct; 14(5): 775–784.*

[4] Sharma et al (2006).

[5] Chainark P, Satoh S, Hirono I, Aoki T, Endo M (2008). **Availability of genetically modified feed ingredient: investigations of ingested foreign DNA in rainbow trout** Oncorhynchus mykiss. Fisheries Science; 74: 380–390.

[6] The following information on glufosinate is all taken from: Watts, M (2008). **Glufosinate-ammonium monograph.** Pesticide Action Network New Zealand. http://www.pananz.net/resources/Div_Loaded_Files/Documents/Glufosinate/Glufosinate%20monograph%2012%20Dec%202008.pdf

[7] http://www.nda.agric.za/doaDev/sideMenu/biosafety/doc/GMO%20permits%20-%202012(no%20marker)up020812.pdf

[8] James, Clive (2011). **Global Status of Commercialized Biotech/GM Crops: 2011**. *ISAAA Brief* No.

43. ISAAA: Ithaca, NY.

[9] Benbrook, C (2009). **Impacts of genetically engineered crops on pesticide use: The first 13 years.** The Organic Centre. http://www.organic-center.org/science.pest.php?action=view&report_id=159

[10] Centre for Food Safety (2012). **Comments to EPA on notice of receipt of applications to register new uses of 2,4-D on enlist AAD-1 Corn and Soybean**

[11] Leslie London, Director, School of Public Health & Family medicine. University of Cape Town. Personal correspondence.

[12] ACB (2012). **How much glyphosate is on your dinner plate? SA's food safety compromised by lack of testing.** http://acbio.org.za/images/stories/dmdocuments/ACB_Glyphosate_Food_Safety_in_SA_July2012.pdf

[13] http://www.unep.org/bpsp/EIA/Case%20Studies/South%20Africa%20(EIA).pdf