ACB's objection to Monsanto's application for commodity import of GM maize for a number of events: herbicide tolerance including for dicamba, as well as pest resistance

MON 87427 x MON 89034 x MIR 162 x MON87419;
MON 87427 x MON 89034 x MON810 x MIR 162 x MON 87411 x MON 87419;
and MON 87427 x MON 87419 x NK603

May 2019

african centre for biodiversity
www.acbio.org.za

On 7 April 2015 the African Centre for Biosafety officially changed its name to the African Centre for Biodiversity (ACB). This name change was agreed by consultation within the ACB to reflect the expanded scope of our work over the past few years. All ACB publications prior to this date will remain under our old name of African Centre for Biosafety and should continue to be referenced as such.

We remain committed to dismantling inequalities in the food and agriculture system in Africa and our belief in peoples' right to healthy and culturally appropriate food, produced through ecologically sound and sustainable methods, and their right to define their own food and agriculture systems.

www.acbio.org.za

PO Box 29170, Melville 2109, Johannesburg, South Africa.
Tel: +27 (0)11 486 1156

Design layout: Adam Rumball, Sharkbuoys Designs, Johannesburg

Contents

Key Concerns

- The three GM varieties have not been adequately characterised at the molecular level, raising concerns for unintended effects that may have environmental or human health impacts.
- While the application clearly specifies that this approval is being sought as these GM products may be present in consignments imported into South Africa, low-level presence can equate to significant, unevenly distributed amounts in large, bulk shipments of grain that have not been approved for cultivation, and requires additional safety assessments.
- Safety assessments have been entirely inadequate to substantiate any claims of safety. No feeding studies on the stacked traits have been performed, and many assumptions on safety have been made that are challenged by independent data showing harm.
- The applicant does not provide sufficient information to confirm a lack of disruption to endogenous maize genes or regulatory sequences.
- The applicant does not provide sufficient information proving absence of novel RNA variants
- A number of claims made in the safety assessment are questionable. These include:
  - The applicant concludes that the three varieties are compositionally equivalent to conventional varieties of maize;
  - The proteins have no structural similarities to known toxins or other biologically active proteins that could cause adverse effects;
  - There is a 'history of safe use';
  - The transproteins are rapidly digested in mammalian gastrointestinal systems; and
  - Lack of mammalian toxicity.
- Despite the limited analysis done by the applicant on the compositional analyses, there were significant differences found, which should trigger further study to explore potential risks, and whether or not they are affected by differing environmental interactions e.g. pesticide applications, or climactic effects that may well exacerbate the unintended effects in the GM varieties.
- No assessment of allergenicity of the whole plant was performed, making it impossible to conclude that there is no risk of allergenicity for any of the stacked varieties.
- The applicant has failed to provide chronic feeding studies on the entire plant, thereby failing to confirm a lack of toxicity.
- Data on use and the effects of processing on residues of maize in the growing area is not specified in the application, severely limiting the food safety assessment.
- Based on the combination effects of the active ingredients identified in this analysis, it is strongly recommended that further specific investigations are carried out in this respect before authorising products. Combinational effects is completely absent from the application.
- The South African government is urged to take heed of international court rulings regarding glyphosate – the wave of glyphosate bans and increasing risks presented to human, animal, and environmental health – and shift away from the use of glyphosate rather than unabatedly exposing the South African public to this toxic chemical in our staple food.
- South Africa continues to place itself at the mercy of international commodity markets, much to the benefit of giant, global grain traders. This is not conducive to the long-term planning required in the South African agricultural sector, nor for providing food security for those who are most vulnerable.

Introduction

The African Centre for Biodiversity (ACB) has played an essential watch-dog role on new GMO permits in South Africa for more than a decade now. Through lodging substantive comments on more than 30 permit applications, we have added substantially to the discourse about the scientific assessment of GMOs, as well as issues of socio-economic impacts and democratic decision-making.

The ACB objects to the approval of thes three stacked maize varieties, which would introduce dicamba-tolerant GM maize grains that may contain residues of toxic chemicals, including dicamba, into the South African food system. It appears that South Africa has not approved the use of MON 87427 as a single event. Previous imports shipments were from the US.

Summary of the Application

The application is for three stacked varieties for commodity clearance, including combinations of the following traits:

MON 87427 utilises a specific promoter and intron combination to drive CP4 EPSPS protein expression in vegetative and female reproductive tissue. This provides for maize lines with tissue selective glyphosate tolerance to facilitate the production of viable hybrid maize seed.

MON 87419 confers tolerance to the herbicides dicamba (3,6-dichloro-2-methoxybenzoic acid) and glufosinate (2-amino-4-(hydroxymethylphosphinyl)butanoic acid). MON 87419 contains a demethylase gene from *Stenotrophomonas maltophilia* that expresses a dicamba mono-oxygenase (DMO) protein to confer tolerance to dicamba herbicide and the phosphinothricin N-acetyltransferase (pat) gene from *Streptomyces viridochromogenes* that expresses the PAT protein to confer tolerance to glufosinate-ammonium, the active ingredient in Liberty® herbicide.

NK603 produces a 5-enolpyruvylshikimate-3-phosphate synthase protein from *Agrobacterium sp*. strain CP4 (CP4 EPSPS), which confers tolerance to glyphosate, the active ingredient in the Roundup® family of agricultural herbicides.

MON 89034 produces two insecticidal proteins that protect against feeding damage caused by European corn borer (Ostrinia nubilalis) and other targeted lepidopteran insect pests. Cry1A.105 is a modified *Bacillus thuringiensis* (Bt) Cry1A protein and Cry2Ab2 is a Bt (subsp. kurstaki) protein.

MON 810 produces the Cry1Ab insecticidal crystal (Cry) protein (*δ-endotoxin*) derived from *Bt subsp. kurstaki (B.t.k.)*. The Cry1Ab protein intends to provide protection from feeding damage caused by European corn borer (ECB).

MIR162 produces the Bt Vip3Aa20 protein, which intends to protect against feeding damage caused by fall armyworm (Spodoptera frugiperda), corn earworm (Helicoverpa zea) and other targeted lepidopteran insect pests. MIR162 also expresses the phosphomannose isomerase (PMI) enzyme from *Escherichia coli*, as a plant selectable marker.

MON 87411 claims protection against corn rootworm (CRW) (Diabrotica spp.) and tolerance to the herbicide glyphosate. MON 87411 contains a suppression cassette that expresses an inverted repeat sequence designed to match the sequence of western corn rootworm (WCR; *Diabrotica virgifera virgifera*). The expression of the suppression cassette results in the formation

of a double-stranded RNA (dsRNA) transcript containing a 240 bp fragment of the WCR Snf7 gene (DvSnf7). Upon consumption, the plant-produced dsRNA in MON 87411 is recognised by the CRW's RNA interference (RNAi) machinery resulting in down-regulation of the targeted DvSnf7 gene leading to CRW mortality. MON 87411 also contains a cry3Bb1 gene that produces a modified Bt (*subsp. kumamotoensis*) Cry3Bb1 protein to protect against CRW larval feeding. In addition, MON 87411 contains the *cp4 epsps* gene from *Agrobacterium sp. strain CP4* that encodes for the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) protein, which confers tolerance to glyphosate, the active ingredient in Roundup® agricultural herbicides.

The Executive council has approved the commodity import of other stacked varieties containing the above-mentioned traits including:
MON 89034 x MIR162 (2017);
MON 87427 x MON 89034 x MIR162 x MON 87411 (2018);
MON 87427 x MON 87460 x MON 89034 x MIR162 x NK603 (2018);
MON 87427 x MON 87460 x MON 89034 x TC1507 x MON 87411 x DAS59122-7 (2018);
MON 87427 x MON 89034 x TC1507 x MON 87411 x DAS59122-7 x MON 87419 (2018);
MON 87427 x MON 89034 x MON 87419 x NK603 (2018).

MON 87419 has only been authorised for cultivation in three countries to date, Brazil, Canada and the United States of America.[1] None of these stacked events has been authorised anywhere else in the world.

While the application clearly specifies that this approval is being sought as these GM products "may be present in consignments imported into South Africa by international grain traders", the ACB maintains that due to the 0% tolerance for adventitious presence, the South African government should not be quick to grant such approvals. Low-level presence can equate to significant, unevenly distributed amounts in large, bulk shipments of grain, which have not been approved for cultivation (Demeke *et al*. 2005). This therefore raises serious concerns around food safety, which are not addressed sufficiently in the application.

Key identified Harms and risks

The combined-trait products detailed above, which are stacked using conventional breeding methods, present a number of potential harms that would have significant threats. Since the application is for commodity import, this objection focuses exclusively on potential harms that the import of these combined-trait products could pose, and outlines the pathways to harm, and the potential risks.

These stacked products include a number of events, which are for herbicide tolerance (**MON 87427, MON 87419, NK603,** and **MON 87411**) as well as pest resistance (**MON 89034, MON 810, MIR162,** and **MON 87411**).

Molecular considerations

Characterising the genetic modification is necessary at the level of the genome to identify the location of the integration site of the transgene and stability of the transgenes, as well as the number of copies of the transgene integrated into the maize genome. Any disturbances at the

1. https://www.isaaa.org/gmapprovaldatabase/event/default.asp?EventID=409&Event={recEvents.EventName}

genomic level could have consequences for the transcriptomic, genomic or metabolomic activity of the plant.

Description of modification before and after modification

The transgenic material has been generated in the laboratory and therefore has no history of safe use in nature. A detailed description of the sequence of the transgenes should therefore be provided.

Description of the three event lines fails to include sequence information to confirm integrity of the transgenic DNA. Alterations in the sequence has the potential to alter the function, expression, and activity of the transgene products, with potentially unintended effects that have implications for food safety. Indeed, the applications fail to report that the inserted transgenic DNA in MON 87419 has a 602 base pair deletion in the integrated DNA, as reported to the US authorities (APHIS, 2015). Independent analysis of data provided to Indian authorities for NK 603 and MON 89034 also found unintended modifications in the inserted transgenic DNA (Then, 2013).

Characterisation of indel

The applicant does not provide any details on the specific location of the transgenes in any of the stacked events. There is no sequence information or description of the flanking genomic DNA provided. The applicant therefore does not provide information to confirm a lack of disruption to endogenous maize genes or regulatory sequences.

The applicant should be required to provide details showing a lack of disruption to the endogenous maize genome.

The CaMV 35S promoter

All three lines use the 35S promoter from the cauliflower mosaic virus (CaMV). Concerns surrounding the use of this promoter include the potential risks associated with the presence of viral gene VI within the promoter sequence, as well as the presence of a recombination hotspot. A 2012 paper raised concerns over the sequence overlap of the CaMV 35S promoter and gene VI, with gene VI potentially being expressed into the P6 protein (Latham *et al.* 2013). A proper retrospective risk assessment on the Gene VI fragment showed that the gene product is toxic to plants probably through, among other pathways, the inhibition of gene silencing, which is a necessary function universal to plants and animals (see later); hence it is also likely to be toxic to animals including humans. The applicant has not mentioned this possibility, let alone checked for, expression of this protein.

The promoter is also documented for carrying a recombination hotspot, which may increase potential for genetic rearrangements and horizontal gene transfer (HGT) (Ho *et al*. 1999). The promoter, contrary to claims by GM producers, is active in human cells and any horizontal transfer to human cells therefore has the potential to disturb human gene expression (Ho, 2013).

T-nos terminator sequence

All three stacked lines carry the nos 3' terminator sequence. Terminator sequences mark the end of the gene, the site where transcription of the gene should terminate. Analysis of this terminator in transgenic plants has shown that this terminator does not reliably terminate transcription, leading to the generation of novel RNA variants. There is no mention of assessing for the absence of novel RNA variants. As EFSA (2009) says:

(...) the data did demonstrate that an RNA species could be detected that likely initiated in the promoter of the NK603 insert and proceeded through the nos 3' transcriptional termination sequence continuing into the maize genomic DNA flanking the 3' end of the insert.

EFSA assumes that only very low levels of proteins are produced from such RNA species and are thus unlikely to be toxic or allergenic. However, such an assumption is yet to be tested.

The applicant should be asked to provide data proving complete absence of novel RNA variants.

Description and characterisation of changes to the transcriptome, proteome and metabolome

The applications fail to mention any profiling techniques that are now routinely employed to assess global changes in gene, protein and metabolite expression.

The latest studies in relation to GM crops reveal that the genetic modification process disrupts endogenous gene expression in the plant, which has the potential to introduce human and environmental risks as well as agronomic disturbances. A new 2019 peer-reviewed study analysed six GM rice lines engineered to express glyphosate tolerance and Bt insecticidal resistance. All six lines had significant alterations in metabolite levels, which were also different in each line, highlighting the lack of predictability of unintended effects caused by the GM process. As such, the authors recommend including global metabolite profiling in risk assessment procedures (Peng *et al*. 2019).

Mesnage *et al*. (2016) used such techniques to analyse proteome and metabolome profiles of NK 603, an event included in two of the applications, detecting altered levels of proteins and metabolites indicative of oxidative stress, alterations in levels of enzymes involved in glycolysis metabolism, as well as TCA cycle involved in energy production. Metabolome alterations also included a 28-fold rise in polyamines, which play multiple roles in cell growth, survival and proliferation; they can be either toxic or protective depending on the context.

Safety assessment

Establishing the food and feed safety of the three stacked varieties is essential considering that maize is not only consumed by humans and animals in South Africa but it is an important staple crop consumed on a daily basis.

Claims made in the safety assessment are questionable, for example, the applicant concludes that:

1. The three varieties are compositionally equivalent to conventional varieties of maize;
2. The proteins have no structural similarities to known toxins or other biologically active proteins that could cause adverse effects;
3. They have a 'history of safe use';
4. The transproteins are rapidly digested in mammalian gastrointestinal systems; and
5. There is a lack of mammalian toxicity.

Compositional analysis

The principle of 'substantial equivalence' for risk assessment is not a risk assessment but an analytical exercise that compares arbitrary comparators of GM crops to any variety or composite of varieties of conventional crops. The comparative tests included in the application do not allow

for detailed and unbiased detection of compositional differences. Independent analyses that employ more sensitive and global measurements highlight this inadequate approach employed by the applicant. For example, Mesnage *et al*. (2016) used more sensitive and global "omics" profiling techniques to assess thousands of components for NK 603, revealing altered protein and metabolite profiles with increased levels of potentially toxic components. Other GM crops have also been shown to be substantially 'non-equivalent' (Peng *et al*., 2019; Abdo *et al*., 2013; Bøhn *et al*., 2014; Agapito-Tenfen *et al*., 2013).

Despite the limited analysis performed by the applicant that measured under 70 components for the three varieties, MON 87427 x MON 87419 x NK603 showed significant differences in six out of 55 components measured (total fat, palmitoleic acid, behenic acid, zinc, vitamin A and vitamin B9 for grain); 14 out of 61 significant differences for MON 87427 × MON 89034 × MON 810 × MIR162 × MON 87411 × MON 87419 (palmitic acid, palmitoleic acid, oleic acid, linoleic acid, linolenic acid, TDF, iron, zinc, vitamin A, vitamin B3, vitamin B6, vitamin E, ferulic acid and p-coumaric acid in grain); and 18 out of 61 components that were significantly altered for MON 87427 × MON 89034 × MIR162 × MON 87419 × NK603 (protein, alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine).

These alterations suggest that there have been unintended effects as a result of the genetic modification process. Such effects should trigger further study to explore potential risks and to assess whether or not they are affected by differing environmental interactions e.g. pesticide applications or climactic effects that may well exacerbate the unintended effects in the GM varieties.

History of safe use

The three GM varieties were developed in a laboratory and thus do not have a 'history of safe use'.

However, first, this does not take into account that many of the transgenic sequences, as described above, have unintended sequence alterations following the genetic modification process. Second, the Bt toxins introduced have been significantly modified from their natural counterparts and as documented by Latham *et al*. (2017), this has the potential to increase their toxicity. Claims that Bt GM crops in the US have been safely consumed lacks scientific basis, due to the lack of labelling of GM products, which prevents any potential analysis of adverse health effects.

Third, MON87411 contains a transgene encoding a dsRNA molecule that is claimed by the applicant to have a history of safe use, based on outdated assumptions from studies published between 2009-2013 that state there is limited uptake of dsRNA molecules in the mammalian digestive system. However, it is now well established that dsRNAs survive the mammalian digestive system, and are even suggested to be bioactive, as reviewed by Nawaz *et al*. (2018).

Allergenicity

No assessment of allergenicity of the whole plant was performed, making it impossible to conclude that there is no risk of allergenicity for any of the stacked varieties.

Only bioinformatics tools were used in the assessment of allergenicity. An eight-amino acid sliding window search was used by the applicant to specifically identify short linear polypeptide matches to known or suspected allergens. The applicant notes that the Codex Alimentarius Commission (2003) recommends that the size of the contiguous amino acid searched should be based on a scientifically justified rationale, and chooses to use eight amino acids in its analysis (Codex, 2003).

The 2001 FAO/WHO consultation on the assessment of possible allergenicity due to GM foods however had suggested moving from eight to six identical amino acid segment searches. Codex (2004) notes: "The smaller the peptide sequence used in the stepwise comparison, the greater the likelihood of identifying false positives, inversely the larger the peptide sequence used, the great the likelihood of false negatives, thereby reducing the utility of comparison". Using six amino acids for comparison would therefore be more precautionary, and in line with the thrust of the Biosafety Act and the Cartagena Protocol on Biosafety, to which South Africa is a Party.

Limitations in the allergenicity analyses is highlighted by studies that have now linked Cry toxins to immunogenic reactions in mammals. Especially Cry1Ac (which shows some similarity to Cry1A.105) is known to enhance immune reactions and able to bind to epithelial cells in the intestine of mice (Vázquez-Padrón *et al*., 1999), Vásquez-Padrón *et al*., 2000). The applicant should therefore provide further detailed experimental data to rule out the potential for the transproteins to induce allergenic responses. In addition, a research paper comparing six amino acids found that Cry2Ab show sequence similarity with known allergenic proteins (Kleter *et al*., 2002).

As described in the molecular characterisation, unintended changes in transgene sequences for MON87419, MON89034 and NK603 have been documented. Whether these alterations were included in the bioinformatics analysis is not clarified.

Survival of GM transproteins following mammalian digestion

The applicant claims that exposure is limited based on data from *in vitro* digestibility assays with all the transproteins that show them to be rapidly digested by gastrointestinal enzymes. However, these protocols prescribed by the WHO/FAO are limited as they do not test a range of pHs despite variability in human stomach pH, with infants generally having a higher pHs. Simulation experiments are of limited relevance to the physiology of the mammalian gut and do not prove a lack of survival of proteins in the digestive tract. Indeed, analysis of human blood samples of pregnant women and their foetal blood supply found 90% of women consuming a standard Canadian diet tested positive for Bt toxins, despite the toxin having been shown in digestibility assays to be rapidly digested in regulatory testing (Aris *et al*., 2011).

Mammalian toxicity data

No toxicity data is presented by the applicant on any of the whole plants in mammalian oral toxicity studies, with only acute oral toxicity performed with microbial versions of individual transproteins. This fails to take into consideration any potential effects of post-translational modifications of the proteins in the whole that may alter the activity and safety of the proteins; potential combinatorial effects of multiple transproteins and any potential chronic effects that would be missed in the acute studies.

Further, feeding studies on MON87427 submitted to EFSA (EFSA, 2015b) show that in sub-chronic 90-day rat feeding studies, there were significant effects observed, including statistically significant higher body weights, lower cholesterol levels in males, increased serum alanine aminotransferase activity in females, and significant difference in organ weights in females including in the brain, ovaries, thyroid/parathyroid and thymus. Without further long-term analysis, the significance of such alterations with regards to mammalian health remains unstudied.

In order to claim a lack of mammalian toxicity, the applicant should provide chronic feeding studies with the whole plant. We urge the authorities to ask the applicant to provide actual toxicity data relevant to these crops, and to further investigate the observed abnormalities already described above and their relevance to human health. Without confirming a lack of toxicity, approval of these crops presents a human health risk to the population of South Africa.

Pesticide toxicity

These traits provide protection from glyphosate, glufosinate, and dicamba. The ACB has made numerous objections based on the use and increased reliance on toxic chemicals, and cocktails of these dangerous chemicals, which has significant impacts on biodiversity (soil, plant, insects, amongst others), and human health (farmers, farm workers, and consumers). The recognition of the health dangers posed by these dangerous pesticides is increasingly being recognised.

These stacked traits are designed to be cultivated in conjunction with these pesticides. For the importation of these products that may enter the South African food system as food, feed or for processing, dangers still exist for consumers who eat these products daily.

The risk assessment fails to consider the sever harm that residues of glyphosate, glufosinate, and dicamba would pose to the South African population. With maize being a staple food in South Africa, the figures provided for by the applicant do not address the high direct consumption patterns of maize in South Africa, and the amount fed to animals.

Herbicide-resistant plants are meant to survive the application of the complementary herbicide while most other plants will die after short time. Thus, for example, residues of glyphosate, its metabolites, and additives to the formulated product might accumulate and interact in the plants. As the publication by Kleter *et al.* (2011) shows, using herbicides to spray genetically engineered herbicide resistant plants does indeed lead to patterns of residues and exposure that need to be assessed in detail. According to a reasoned legal opinion drawn up by Kraemer (2012), residues from spraying with complementary herbicides have to be taken into account in the risk assessment of genetically engineered plants, from a regulatory point of view.

Dicamba

Dicamba is in the benzoic acid herbicide family, similar in structure and mode of action to phenoxy herbicides like 2,4-D. Like phenoxy herbicides, dicamba mimics auxins, a type of plant hormone, and causes abnormal growth by affecting cell division. Dicamba acts systemically in plants (throughout the entire plant) after it is absorbed through leaves and roots. It is easily transported throughout the plant, and also accumulates in new leaves. Dicamba also inhibits an enzyme found in the nervous system of most animals, acetylcholinesterase. Inhibition of acetylcholinesterase causes a neurotransmitter, acetylcholine, to accumulate and prevents smooth transmission of nerve impulses. In addition, dicamba inhibits the activity of several enzymes in animal livers that detoxify and excrete foreign chemicals. Studies have shown dicamba to be linked to neurotoxicity, chronic toxicity, effect reproduction, induction of mutagenesis, and it act as a carcinogen.[2] Regulatory data suggests dicamba can exert reproductive/developmental toxicity in mammals. For example, multi- generation reproductive studies reported increased abortions, decreased food consumption and weight gain, with offspring displaying skeletal abnormalities, including irregular ossification of nasal bones.[3]

Dicamba metabolites have also been linked to toxicity, with one – DCSA – described by EFSA as a weak clastogen that induces abnormalities in chromosomes (EFSA Journal 2013;11(10):3440).

2. See U.S. EPA. Office of Pesticides and Toxic Substances. 1984. Summary of results of studies submitted in support of the registration of dicamba. Washington, D.C. (Sept. 29.) 18. California Dept. of Food and Agriculture. Medical Toxicology Branch. 1990. Summary of toxicology data: Dicamba. Sacramento, CA. (Sept. 17.); Beasley, V.R. 1991. 2,4-D toxicosis I: A pilot study of 2,4-dichlorophenoxyacetic acid- and dicambainduced myotonia in experimental dogs. Vet Hum. Toxicol. 33(5):435-440; Cox, C., 1994, Dicamba, *Journal of Pesticide Reform*, 14(1): 30-35
3. EFED Re-registration Chapter For Dicamba/Dicamba Salts; U.S. Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Office of Pesticide Programs, U.S. Government Printing Office: Washington, DC, 2005.

There is no information provided on residues from **Dicamba** spraying, or interactions between the residues from spraying, and plant biochemistry and metabolism. No information is provided on the effect of processing on the nature of dicamba residues. The food safety assessment is incomplete and inadequate to make a decision. **Data on use and the effects of processing on residues of maize in the growing area is not specified in the application, severely limiting the food safety assessment.**

Glyphosate

Glyphosate toxicity has been widely documented in both independent and industry data. Most recently Bayer, formally Monsanto, lost two high profile court cases in the US where Monsanto was found guilty of not warning of the cancer risks of glyphosate herbicides that had resulted in two people suffering from non-Hodgkins lymphoma (NHL). There are a further 11,200 cases pending and it is now estimated that Bayer could be liable to pay US$ 30 billion in compensation (Sustainable Pulse, 2019). These court cases have also revealed aggressive PR strategies by Monsanto to cover up evidence of cancer links, including ghost-writing of scientific papers and pushing favourable studies (McHenry, 2018), in efforts to delegitimise the 2015 decision by the International Agency for Research on Cancer (IARC) to classify glyphosate as a Group 2A probable human carcinogen, spending an estimated US$ 17 million in a single year.

In recent days a draft US federal report from the Agency for Toxic Substances and Disease Registry, tied to the Centres for Disease Control and Prevention (CDC), echoed the IARC decision, acknowledging a link to NHL (ATSDR, 2019). These recent developments have rightly prompted numerous national and regional bans or restrictions on the herbicide, including a complete ban on production and import in Vietnam (Reuters, 2019) and Malawi (Sustainable Pulse, 2019b), ban on sales in France, and numerous regional bans across European, Asian and Latin American nations, and national bans on home use. Glyphosate has also been linked to reproductive and developmental toxicity, disruption of the microbiome, and liver disease at legally permitted levels for human exposure.

Studies have shown glyphosate is not only a food safety and environmental issue from direct application but is also taken up taken by the plants themselves, present in the stem, leaves, and beans, traceable in humans and in animals, as well as detectable even after processing, in a case of soybean oil (Kruger *et al.* 2014). **Data on use and the effects of processing on residues of maize in the growing area is not specified in the application, severely limiting the food safety assessment.**

Studies indicate the correlation between glyphosate applications and a range of human diseases and deaths (Swanson *et al*. 2014), including strong and significant correlations between use of glyphosate and cancers including NHL, birth defects, and reproductive problems. Lab data has also shown a cause and effect relationship between legal levels of glyphosate and non-alcoholic fatty acid liver disease, raising concerns regarding the legal limits that have been set for glyphosate, which are based on inadequate and outdated industry data.

Correlations between glyphosate use and other health problems in the US have also been made, including deaths due to hypertension, strokes, obesity, diabetes, lipoprotein metabolism disorders, renal disease and failure, intestinal infection, senile dementia, Alzheimer's, Parkinson's disease, and increased prevalence of diabetes, inflammatory bowel disease, and autism (Swanson *et al.* 2014).

Glyphosate has been shown to induce endocrine disruption and to disrupt the ability of animals (including humans) to detoxify xenobiotics, intensifying accumulation of the variety of chemicals humans are exposed to (Samsel and Seneff, 2013a and b). It has also been linked to changes in cell functions, necrosis in cells, and neurotoxic effects in brain, kidney hepatic, testis and Sertoli cells (Cattani *et al.* 2014). While this does not indicate causation, there is significant

evidence to support increased caution in using the highly prevalent herbicide. A recent epigenetic study also pointed to the fact that glyphosate induces transgenerational inheritance of disease and germline epimutations. The observed transgenerational pathologies included prostate disease, obesity, kidney disease, ovarian disease, and birth abnormalities (Kubstad *et al.* 2019). Studies suggest it bio-accumulates in animal and human bodies, raising concerns over the long-term impacts of consuming maize products contaminated with glyphosate residues. As we have recently reported, there are no established safe levels of glyphosate. Consistently, peer-reviewed data has shown that legal levels can induce liver disease in lab animals.

There is a high likelihood that glyphosate would be used, with residues on the plant and product, and with a high likelihood that it would be consumed by animals and humans as feed and food, this on its own could cause significant risk to humans.

The South African government is urged to take heed of international court rulings and the wave of glyphosate bans, which is based on increasing risks presented to human, animal, and environmental health, and shift away from the use of glyphosate, rather than unabatingly continue to expose the South African public to toxic chemicals in our staple food.

Glufosinate

This broad-spectrum contact herbicide is used to control a wide range of weeds after the crop emerges or for total vegetation control on land not used for cultivation. Glufosinate herbicides are also used to desiccate (dry out) crops before harvest. Glufosinate is a short name for the ammonium salt, glufosinate ammonium. It is derived from phosphinothricin, a natural microbial toxin isolated from two species of Streptomyces fungi. Glufosinate is a phosphorus-containing amino acid that inhibits the activity of the enzyme, glutamine synthetase, which is necessary for the production of the amino acid glutamine and for ammonia detoxification. The application of glufosinate leads to reduced glutamine and increased ammonia levels in the plant's tissues. This causes photosynthesis to stop and the plant dies within a few days. Studies show that Glufosinate-ammonium competitively inhibits glutamine synthetase in mammals. While even at high (sub-lethal) doses, glutamate, ammonia and glutamine levels in brain, liver and kidney tissues were unaffected. The compound showed slight to moderate acute oral toxicity in rats, mice and dogs.

Glufosinate ammonium structurally resembles glutamate, a typical excitatory amino acid in the central nervous system. The US EPA estimated the chronic reference dose (cRfD) for human health effects of glufosinate, using rat suchronic and chronic toxicity studies, revealing an inhibition of brain glutamate synthetase associated to developmental neurotoxic effects (at >6 mg/kg bw/d) (EPA, 2012). It is recognised that excess release of glutamate results in the death of nerve cells in the brain. In mammals, both glufosinate and the surfactant, AES, are rapidly absorbed through the gut (FoE, 2001). Ingestion of glufosinate affects the nervous system and evidence of neurotoxicity has been found in most species of laboratory animals exposed to glufosinate (FoE, 2001).

A total of three long-term toxicity and carcinogenicity studies have been reported in the draft assessment report (DAR) provided by the designated rapporteur Member State of the EU-Commission (DAR, 2004). One study showed that the administration of glufosinate provoked a dose-dependent decrease in the incidence of pituitary adenomas in males (Schmid, 1998).

Toxic effects due to glutamate-mimicking properties

Mechanistic insight was lacking to explain reproductive toxicity effects but reduced glutamine synthetase activity may be involved. Evidence of glutamine synthetase inhibition came from neurotoxicity studies, showing a reduction in the activity of this enzyme in male rat liver (Hamann *et al*. 2000). A 10% reduction in glutamine synthetase activity is a marker of adverse effects on brain biochemistry and behaviour (EFSA, 2005).

Since glufosinate is a structural analogue of glutamate, potential disturbances of glutamate metabolism by this compound has been one of the more intensely studied toxicological endpoints. Glufosinate has been shown to bind to NMDA excitatory receptors in binding assays, and as a result increase production of nitric oxide (Lantz etal, 2014). Several studies have reported the ability of glufosinate to disrupt neurogenesis. Mice exposed to intraperitoneal injections of 2.5-10 mg/kg body weight, three times a week for 10 weeks, showed dose-dependent structural changes in the hippocampus and somatosensorial cortex (Meme *et al.* 2009).

Another neurobehavioural study performed on C57BL/6J mice treated over a 10-week period with glufosinate-ammonium administered three times a week at doses of 2.5, 5 and 10 mg/kg (Calas, 2008) showed structural modifications in the hippocampus at the two higher doses, as well as a modification of glutamate metabolism, reflected by an increased GS activity. This suggests that the exposure to glufosinate-ammonium could lead to mild memory impairments. In this case, the increased GS activity (while it was decreased in the other experiments) could be due to the ammonium component of the pesticide, which can cause hyperammonemia and stimulate GS activity (Suarez *et al.* 2002). Additionally, a radial-maze test performed in the same study (Calas, 2008) showed that glufosinate-ammonium led to a deficit in spatial learning when administered at 5 and 10 mg/kg. More recently, a study showed that mice perinatally exposed to subregulatory doses of glufosinate-ammonium (0.2-1 mg/kg bw/d), presented with impaired neuroblast proliferation and neurogenesis, as well as an abnormal migration of neural stem cells to the olfactory bulb (Herzine *et al.* 2016). Whole brain transcriptomics data showed that the expression of genes regulating the cytoskeleton, cell proliferation and cell migration were affected. A follow-up investigation revealed that such disturbances were associated with autistic symptoms (Laugeray *et al.* 2014).

Residues in food are an area of concern, especially when glufosinate is used as a pre-harvest desiccant. The WHO/FAO recommended acceptable daily intake (ADI) for glufosinate is 0.02 mg/kg body weight. In the United Kingdom, it was reported that when wheat grain containing residues was turned into flour, 10%-100% of the residue was retained. Residue levels in bran were 10%-600% of those in grain. In addition, it was found that the use of glufosinate as a herbicide and/or a desiccant in potato crops can lead to residues in the tubers in the order of 0.1 mg/kg. Residues of the metabolite MPPA-3 were found in potato tubers 77 days after treatment, 0.07 mg/kg were detected after a single treatment, and 0.24 mg/kg following double rate treatment. A report produced by the UK Pesticides Safety Directorate in 1998 stated that it was probable that GM crops would receive at least two applications of glufosinate. **Data on use and the effects of processing on residues of maize in the growing area is not specified in the application, severely limiting the food safety assessment.**

Combined effects

There are known **combined effects** of glyphosate with both dicamba and glufosinate. These effects are, with dicamba: genotoxicity, an increased number of dead foetuses, altered thymus weight and, for glyphosate-based herbicides and dicamba based herbicides, reduced susceptibility to antibiotic drugs (Kurenbach *et al*. 2018). Consumers can be exposed to a combination of these substances if they are found together as residues in food. Combined effects are not assessed during the authorisation process for the genetically modified crops under scrutiny.

Pesticide active ingredients are never applied alone but always in their commercial formulations. These formulations are not tested extensively for authorisation. For one group of ingredients in glyphosate-based herbicides, the tallow amines: "a higher toxicity was observed on all endpoints investigated compared to glyphosate" (EFSA, 2015). There is no information regarding the residues in plants and livestock. Based on the combination effects of the active ingredients identified in this analysis, it is strongly recommended that further specific investigations are carried out in this respect before authorising products that may contain combined residues of

the above-mentioned herbicides. This should also include the commercial formulations and analyses of residues.

Based on the above, and due to the lack of scientific evidence and scientific consensus, and the lack of information provided by the applicant on application of pesticides and pesticide residues, we maintain that the food safety assessment is sorely lacking and needs further information in order for the application to be assessed. Based on the known evidence, there are potential pathways to harm, as detailed above. **If residues do persist, they would enter the food chain and potentially cause significant harm to human and animal health, posing a potentially severe risk that should be avoided.** Effective monitoring of residues would need to be done, which is impossible and unlikely to occur.

Therefore due to the high likelihood of the imports of these products resulting in consumption by humans and/or animals, it is necessary to gain an understanding of the half-life of all of the individual chemicals, and any combined effect: from their use, application, the environment where they are applied (and any effect this may have on residue half-lives), as well as the effect of transportation, of processing, and consumption of these by adults, or children, in whole or processed forms. **This information is completely absent from the application.**

Conclusions

Based on well-established evidence of pesticide toxicity, and evidence of risks to human health of the GM crop varieties, as well as a lack of critical data to support claims of safety, we urge the South African authorities to reject the approval of these crops. Promoting the unsustainable system of industrialised chemical agriculture is a major contributing factor to climate change and biodiversity loss, which has recently been established to be threatening the extinction of a million species. A transition towards sustainable agroecological approaches, as recommended by the latest IPBES report (2019), is an urgent requirement to protect food security, biodiversity and the fabric of human societies.

Bulk shipments to the world's largest maize importing region may offer temporary respite for some commercial farmers, but is this a viable long-term strategy? The scale of production and transportation that would be required to compete with the global maize giants would squeeze margins even tighter. This would, no doubt, benefit the global grain traders but what of farmers and rural communities in South Africa? South Africa is putting itself at the mercy of international commodity markets, which is hardly conducive to the long-term planning required in the South African agricultural sector, or for providing food security for those who are most vulnerable.

We can begin this transition by taking a stance against this foreign-imposed, corporate destruction of our food here in South Africa.

References

Abdo EM, *et al.* 2013. Chemical Analysis of BT corn "Mon-810: Ajeeb-YG ®" and its counterpart non-Bt corn "Ajeeb". IOSR *Journal of Applied Chemistry*, 4: p. 55-60

APHIS., 2015. Petition for the Determination of Nonregulated Status for Dicamba and Glufosinate Tolerant MON 87419 Maize. https://www.aphis.usda.gov/brs/aphisdocs/15_11301p.pdf

ATSDR., 2019. Toxicological Profile for Glyphosate. Draft for Public Comment. https://www.atsdr.cdc.gov/toxprofiles/tp214.pdf

Agapito-Tenfen, S.Z., *et al*. 2014. Effect of stacking insecticidal cry and herbicide tolerance epsps transgenes on transgenic maize proteome. *BMC plant biology* 14:p 346.

Aris, A. and S. Leblanc, 2011. Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada. *Reprod Toxicol,* 31(4): p. 528-33.

Benbrook, C.M., 2016. Trends in glyphosate herbicide use in the United States and globally. E*nvironmental Sciences Europe*, 28(1): p. 3.

Bremmer, J.N. andLeist, K.-H., 1997. *Disodium-N-acetyl-L-glufosinate; AE F099730 - Hazard evaluation of Lglufosinate produced intestinally from N-acetyl-L-glufosinate. Hoechst Schering AgrEvo GmbH, Safety Evaluation Frankfurt. TOX97/014. A58659. Unpublished.* (see FAO publication on https://web.archive.org/web/20060101000000*/http://www.fao.org/ag/agp/agpp/pesticid/jmpr /Download/98/glufosi3.pdf). 1997.

Bonny, S., 2016. Genetically Modified Herbicide-Tolerant Crops, Weeds, and Herbicides: Overview and Impact. *Environ Manage*, 2016. 57(1): p. 31-48.

Calas, A.G., *et al*. 2008. Chronic exposure to glufosinate-ammonium induces spatial memory impairments, hippocampal MRI modifications and glutamine synthetase activation in mice. *Neurotoxicology*, 2008. 29(4): p. 740-7.

Cattani, D., de Liz Oliveira Cavalli, V.L., Heinz Rig, C.E., Dominguez, J.T., Dal-Cim, T., Tosca, C.I., Mena Barreto Silva, F.R. and Zamoner, A,. 2014. Mechanisms underlying the neurotoxicity induced by glyphosate-based herbicide in immature rat hippocampus: Involvement of glutamate excitotoxicity. Toxicology. 2014 Mar 15;320C:34-4;

Codex Alimentarius Commission. 2003. Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants. Codex CAC/GL 45-2003:1-13.

Codex Alimentarius Commission. 2004. Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants (CAC/GL 45-2003). Rome: Food and Agriculture Organization of the United Nations and World Health Organization.

DAR, G., 2004. Initial risk assessment provided by the rapporteur Member State Sweden for the existing active substance GLUFOSINATE (based on the variant glufosinate-ammonium) of the second stage of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC. Volume 3, Annex B, B.6 January 2004. 2004.

Demeke, T., Perry, D.J., and Scowcroft, W.R., 2005. Adventitious presence of GMOs: scientific overview for Canadian grains. Canadian Grain Commission.

EFSA 2015: Statement of EFSA on the request for the evaluation of the toxicological assessment of the co-formulant POE-tallowamine. EFSA Journal 2015;13(11):4303, 13 pp.

EFSA 2015b. Scientific Opinion on the application (EFSA-GMO-BE-2012-110) for the placing on the market of tissue-selective herbicide-tolerant genetically modified maize MON 87427 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto.

EFSA, 2009.. Scientific Opinion of the Panel on Genetically Modified Organisms on applications (EFSA-GMO-NL-2005-22 and EFSA-GMO-RX-NK603) for the placing on the market of the genetically modified glyphosate tolerant maize NK603 for cultivation, food and feed uses and import and processing, and for renewal of the authorisation of maize NK603 as existing product. The EFSA Journal 2009, 1137 Available online: https://www.efsa.europa.eu/en/efsajournal/pub/1137

EFSA, 2005. Conclusion regarding the peer review of the pesticide risk assessment of the active substance glufosinate. *EFSA Journal*, 2005. 3(4): p. 27r-n/a

Fabian, D., *et al*. 2011. The effect of herbicide BASTA 15 on the development of mouse preimplantation embryos in vivo and in vitro. *Toxicol In Vitro*. 25(1): p. 73-9.

Garcia, A.M., *et al.* 1998. Paternal exposure to pesticides and congenital malformations. *Scand J Work Environ Health*, 1998. 24(6): p. 473-80.

Hamann H.-J., W.K., Luetkemeier H., Biedermann K., Werner H.- J., Bieler G., *Repeat dose neurotoxicity study in the rat including water maze, functional observation battery and brain-, liver- and kidney-glutamine synthetase enzyme activities Glufosinate-ammonium and N- acetyl-L-glufosinate disodium Code: AE F039866 00 TK50 A133, Generated by: RCC Umweltchemie AG, Itingen, CH; Toxicology Document No: C008991 (unpublished report).* 2000.

Heap, I., 2014. Global perspective of herbicide-resistant weeds. *Pest Manag Sci*, 70(9): p. 1306-15.

Herzine, A., *et al.* 2016. Perinatal Exposure to Glufosinate Ammonium Herbicide Impairs Neurogenesis and Neuroblast Migration through Cytoskeleton Destabilization. *Front Cell Neurosci,*. 10: p. 191.

Ho MW., 2013. Natural versus Artificial Genetic Modification. *Entropy*, 15, p. 4748-4781

Ho MW, *et al*. 1999. Cauliflower Mosaic Viral Promoter – A recipe for Disaster? *Microbial Health and* 1999, 11, p 194-197

IPBES., 2019. Nature's Dangerous Decline 'Unprecedented'; Species Extinction Rates 'Accelerating'. https://www.ipbes.net/news/Media-Release-Global-Assessment
Kleter, G.A., Unsworth, J.B., Harris, C.A. (2011) The impact of altered herbicide residues in transgenic herbicide-resistant crops on standard setting for herbicide residues. Pest Managment Science, 67(10): 1193-1210.
Kraemer, L., 2012. The consumption of genetically modified plants and the potential presence of herbicide residues, legal dossier compiled on behalf of Testbiotech, http://www.testbiotech.de/sites/default/files/Legal_Dossier_Kraemer_Pesticide_RA_PMP.pdf Kramarz.
Kruger, M., Schledorm, P., Schödi, W., Hoppe, H.W., Lutz, W., and Shehata, A.A., 2014. Detection of glyphosate residues in animals and humans. *Journal of Environmental and Analytical Toxicology*, 4(2):210-215
Kubsad, D., Nilsson, E.E., King, S.E., Sadler-Riggleman, I., Beck, D., and Skinner, M.K., 2019. Assessment of glypjosate induced epigenetic transgenerational inheritance of pathologies and sperm epimutations: generational toxicology, *Nature: Scientific Reports*, 9.
Kurenbach, B, Hill, A.M., Godsoe, W., van Hamelsveld, S, and Heinemann, J.D., 2018. Agrochemicals and antibiotivs in combination increase antibiotic evolution. PeerJ 6:e5801
Lantz, S.R., *et al*. 2014. Glufosinate binds N-methyl-D-aspartate receptors and increases neuronal network activity in vitro. *Neurotoxicology*. 45: p. 38-47.
Latham J & Wilson A., 2013. Potentially dangerous virus gene hidden in commercial GM crops. *Sci. Soc*. 57, p. 4–5.
Laugeray, A., *et al*. 2014. Pre- and postnatal exposure to low dose glufosinate ammonium induces autism-like phenotypes in mice. *Front Behav Neurosci*, 2014. 8: p. 390.
Markets, M.a., 2017. Glufosinate Market by Crop Type (Genetically Modified Crops, Conventional Crops), Form (Liquid Formulation, Dry Formulation), Application (Agricultural, Non Agricultural), and Region - Global Forecast to 2022.
Markets, M.a., 2017. Herbicides Market by Type (Glyphosate, 2, 4-D, Diquat), Crop Type (Cereals & Grains, Oilseeds & Pulses, Fruits & Vegetables), Mode of Action (Non-selective, Selective), and Region - Global Forecast to 2022.
Markets and Markets, 2016 Dicamba Herbicide Market by Crop Type (Cereals & Grains, Oilseeds & Pulses, and Pastures & Forage Crops), Formulation (Acid and Salt), Physical Form (Dry and Liquid), & Time of Application (Pre- and Post-Emergence) - Global Forecast to 2022http://www.marketsandmarkets.com/Market-Reports/dicamba-herbicide-market-230485770.html
McHenty, LB., 2018. The Monsanto Papers: Poisoning the scientific well. Int J Risk Saf Med. 29(3-4):193-205. doi: 10.3233/JRS-180028.
Meme, S., *et al*. 2009. MRI characterization of structural mouse brain changes in response to chronic exposure to the glufosinate ammonium herbicide. *Toxicol Sci*, 2009. 111(2): p. 321-30.
Mesnage R. *et al*. 2017. Multiomics reveal non-alcoholic fatty liver disease in rats following chronic exposure to an ultra-low dose of Roundup herbicide. *Sci Rep*. 9;(7):39328.
Mesnage R., 2016. An integrated multi-omics analysis of the NK603 Roundup-tolerant GM maize reveals metabolism disturbances caused by the transformation process. *Scientific Reports*, 6: 37855.
Mesnage, R., *et al*. 2015. Laboratory Rodent Diets Contain Toxic Levels of Environmental Contaminants: Implications for Regulatory Tests. *PLoS One*. 10(7): p. e0128429.
Nawaz MA *et al*. 2019. Addressing concerns over the fate of DNA derived from genetically modified food in the human body: A review. *Food Chem Toxicol*. 124:p 423-430
Peng, C., *et al*. 2019. Effect on metabolome of the grains of transgenic rice containing insecticidal cry and glyphosate tolerance epsps genes. *Plant Growth Regulation*. 88: p 1-7
Reuters., 2019. U.S. criticizes Vietnam ban of glyphosate herbicide imports. https://www.reuters.com/article/us-usa-vietnam-glyphosate/us-criticizes-vietnam-ban-of-glyphosate-herbicide-imports-idUSKCN1RN2F4
Samsel, A. and Seneff, S., 2013a. Glyphosate's suppression of cytochrome P450 enzymes and amino acid biosynthesis by the gut microbiome: pathways to modern diseases. Entropy, 15(4): 1416- 1463;
Samsel, A. and Seneff, S., 2013b. Glyphosate, pathways to modern diseases II: Celiac sprue and gluten intolerance. Interdisciplinary Toxicology, 6(4): 159-184.
Schmid H., K.B., Luetkemeier H., Westen H., Biedermann K., *Oncogenicity study in rats Glufosinate-ammonium substance technical Code: Hoe 039866 00 ZD96 0001. Generated by: RCC Umweltchemie AG, Itingen, CH; Research & Consulting Company Ltd, CH; Biological Research Laboratory Ltd.; EPS (U.K.); Experimental Pathology Services AG; Document No: A67303 GLP / GEP (unpublished report)*. 1998.
Suarez, I., G. Bodega, and B. Fernandez, 2002. Glutamine synthetase in brain: effect of ammonia. *Neurochem Int*, 2002. 41(2-3): p. 123-42.
Sustainable Pulse., 2019. US Legal Claims over Glyphosate Health Damage Set to Reach $31 Billion. https://sustainablepulse.com/2019/04/12/us-legal-claims-over-glyphosate-health-damage-set-to-reach-31-billion/#.XLRrlKeZO_V
Swanson., N.L., Leu, A., Abrahamson, J., and Wallet, B., 2014. Genetically engineered crops, glyphosate and the deterioration of health in the United States of America. *Journal of Organic Systems*, 9(2):6-37
Then C., 2013. Analysis of the data submitted by Monsanto to the Indian authorities on genetically engineered maize MON89034 x NK603. Testbiotech.
Vazquez-Padron RI, *et al*. 1999. Intragastric and intraperitoneal administration of Cry1Ac protoxin from Bacillus thuringiensis induces systemic and mucosal antibody responses in mice. *Life Sciences*, 64, p. 1897-1912
Vazquez-Padron RI, *et al*. 2000. Cry1Ac Protoxin from Bacillus thuringiensis sp. Kurstaki HD73 binds to surface proteins in the mouse small intestine. *Biochem. and Biophy.s Research Comm*., 271,p. 54-58