

**PRESENCE OF GLYPHOSATE IN FOOD PRODUCTS IN SOUTH
AFRICA OF WHICH MAIZE OR SOYBEAN IS THE PRIMARY
CONSTITUENT**

BJ Koortzen

February 2017

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By

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Magister Medical Scientiae (Human Molecular Biology)**

In the Faculty of Health Sciences

Department of Haematology and Cell Biology

University of the Free State

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February 2017

Bloemfontein

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DECLARATIONS

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LIST OF SCIENTIFIC ABBREVIATIONS AND ACRONYMS

°C	Degree Celsius
%	Percentage
µg	Microgram
µL	Microlitre
ADI	Acceptable daily intake
AMPA	Aminomethylphosphoric acid
cAMP	Cyclic adenosine monophosphate
CDK1	Cyclin-dependant kinase 1
CTAB	Cetyltrimethylammonium bromide
dd	Double distilled
DNA	Deoxyribonucleic acid
EDTA	Ethylenediamine tetra acetic acid
ELISA	Enzyme-linked immunosorbent assay
EPSPS	5-Enolpyruvylshikimate-3-phosphate synthase
<i>et al.</i>	Et alia (and others)
g	Gram
GM	Genetically modified
GMO	Genetically modified organism
ha	Hectare
HCl	Hydrochloric acid

HepG2	Hepatocellular carcinoma cell line
HMG	High Mobility Group gene
HT	Herbicide tolerant
JAr	Human choriocarcinoma cell line
kg	Kilogram
L	Litre
LOD	Limit of detection
LOQ	Limit of quantification
m	Metre
M	Molar
MCL	Maximum contaminant level
mg	Milligram
mL	Millilitre
mM	Millimolar
mm ²	Square millimetre
MRL	Maximum residue limit
N	Normal
NaCl	Sodium chloride
NaOH	Sodium hydroxide
ND	Not detected
ng	Nanogram

List of scientific abbreviations and acronyms

nm	nanometre
NOAEL	No observed adverse effect level
NT	Not tested
PCR	Polymerase chain reaction
pH	Percentage hydrogen
POEA	Polyethoxylated tallow amine
ppb	Parts per billion
ppm	Parts per million
R ²	Coefficient of determination
RNAse	Ribonuclease
rpm	Revolutions per minute
SB	Sodium borate
TE	Tris EDTA
UV	Ultraviolet
V	Volt
www	World wide web

**LIST OF ABBREVIATIONS AND ACRONYMS FOR ORGANIZATIONS,
INSTITUTIONS AND/OR AUTHORITIES**

EFSA	European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organisation of the United Nations
USA FDA	United States of America Food and Drug Administration
IARC	International Agency for Research on Cancer
ISAAA	International Service for the Acquisition of Agri-biotech Applications
JMPR	Joint FAO/WHO Meeting on Pesticide Residue
NAMC	National Agricultural Marketing Council SA
NPIC	National Pesticide Information Centre
PMRA	Pest Management Regulatory Agency
PRiF	Expert Committee on Pesticide Residues in Food
SACPA	South African Consumer Protection Act
SA DAFF	South African Department of Agriculture, Forestry and Fisheries
SA DOH	South African Department of Health
SANBI	South African National Biodiversity Institute
UK	United Kingdom
UN	United Nations
USA	United States of America
USA EPA	United States of America Environmental Protection Agency
WHO	World Health Organisation

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PREFACE

Genetically modified (GM) crops are extensively planted around the world with more than 181 million hectares cultivated in 2015. GM crops are considered to have made a positive contribution to agriculture since their introduction in 1996, especially in terms of crop management. The major GM crops are canola, cotton, maize and soybean that are predominantly engineered to be insect resistant and/or herbicide tolerant. South Africa is considered a major GM crop producing country and planted approximately 2.3 million hectares of GM crops in 2015. Of these crops, approximately 75% (1.73 million ha) was herbicide tolerant. The major herbicide tolerant crops planted in South Africa are maize (approximately 1.2 million ha) and soybean (approximately 508,000 ha).

The predominant herbicide used on herbicide tolerant crops is glyphosate. Currently glyphosate is the most widely used herbicide in the world. In 2015, the World Health Organisation International Agency of Research on Cancer (IARC) changed the classification of glyphosate from “possibly carcinogenic to humans” to “probably carcinogenic to humans”. The IARC report, although extensive and in depth, was highly criticised by the agricultural industry. The findings of the IARC on glyphosate differ from regulatory authorities in Europe and the United States of America as well as other international bodies who consider glyphosate safe.

There are several possible reasons why the IARC has reached a different conclusion on the safety of glyphosate compared to other internationally recognised bodies:

- The IARC only considered documents on the safety of glyphosate that are available in the public domain. Compared to this, other bodies have also taken proprietary documentation provided by the herbicide developer and not available in the public domain into account.
- The IARC considered the safety of glyphosate in formulation, which includes surfactants. Compared to this, regulatory bodies assessed the safety of pure glyphosate and not in formulation.

- It should be noted that the safety assessment of glyphosate and glyphosate tolerant crops is evaluated separately and not in combination by regulatory authorities.

Since the commercialization of herbicide tolerant crops, independent research has generated previously unknown information regarding the application of glyphosate on these crops:

- Glyphosate is present in the grain of herbicide tolerant crops treated with glyphosate.
- Glyphosate is not removed from food during processing.
- Low concentrations of glyphosate in formulation have been found to have genotoxic effects in mammalian cells *in vitro*.

Considering that maize is a major staple and soybean an important source of protein, the safety of glyphosate is an issue of great importance in South Africa. However, before any informed discussion can take place on the safety of glyphosate, we need to know the extent of its presence in the food chain in South Africa, since South Africa predominantly produces glyphosate tolerant maize and soybean. Thus, the aim of this study was to test food products in South Africa containing maize and/or soybean as a primary constituent for glyphosate. The food products were purchased from all major retail stores based on their ingredient list. For ethical reasons no brand names are mentioned in this dissertation also taking into account that the controversy surrounding the safety of glyphosate remains unresolved.

It is important to note that this dissertation does not intend to assess the safety of glyphosate but determine whether glyphosate is present in the South African food chain. Care has been taken to present arguments in this dissertation as scientifically as possible with no intention to motivate either for or against the use of glyphosate. To achieve consistency, glyphosate concentrations (either in mg/kg or mg/L) were converted to mg/kg (by using the density of glyphosate 1.75 kg/L) to make data comparison between different studies easier.

This dissertation consists of 6 chapters, including a literature review (chapter 1), research aim and methodology (chapter 2), results and discussion for glyphosate content in food products (chapter 3), results and discussion in terms of compliance with GM labelling in South Africa (chapter 4), limitations of the study (chapter 5), as well as a conclusion (chapter 6). The literature review presents the literature regarding glyphosate, its safety assessment and its presence in HT crops, processed food as well as in animals including humans. Chapter 2 includes the research aim and methodology used. Chapter 3 includes the results and discussion for the level of glyphosate present in the maize and soybean food products. Since the data was available, it was used to determine compliance to GM labelling in terms of the Consumer Protection Act (2008) that mandates GM labelling in South Africa. Chapter 4 includes the results and discussion in terms of compliance with GM labelling requirements in South Africa. Chapter 5 includes the limitations of the study. The final chapter (Chapter 6) presents the conclusions over the presence of glyphosate in the South African food chain as well as compliance to mandatory GM labelling. Following chapter 6 there is a summary of the dissertation.

CHAPTER 1: LITERATURE REVIEW

1.1 Introduction to genetically modified organisms

A genetically modified organism (GMO) is classified by the World Health Organisation (WHO) as “an organism wherein genetic material has been reformed in a way that does not occur naturally by mating and/or natural recombination” (WHO, 2002). GMOs are developed through genetic engineering which entails manipulating an organism’s genetic material by inserting one or more genes or DNA sequences, or by altering one or more bases of the organism’s genetic code (Paoletti *et al.*, 1996). A GMO has altered DNA, which can either encode for a new protein or modify the function of an existing protein (Paoletti *et al.*, 1996). Genetic modification allows the addition of new properties or “traits” that are not naturally present in the organism (König *et al.*, 2004).

The gene inserted into the GMO, also known as the transgene, forms part of a transgene cassette. The transgene cassette contains a promoter, the gene of interest and a terminator (Robinson *et al.*, 2000; Smale and Kadonaga, 2003; Ralston and Shaw, 2008; Lievens *et al.*, 2015). The DNA sequence of the promoter, gene of interest and the terminator can be sourced from various organisms, including bacteria, viruses, plants, fungi and animals (Chawla, 2002). Two methods are commonly used to insert the transgene cassette into the host cell genome (Hansen and Wright, 1999). The first method is particle bombardment which entails the coating of gold or tungsten nanoparticles with the gene cassette (Klein *et al.*, 1987; Segelken, 2010). The coated nanoparticles are then shot into the nucleus of the host cell at high velocity allowing the transgene to be incorporated into the genome of the cell (Klein *et al.*, 1987; Segelken, 2010; Hansen and Wright, 1999). The second method involves the infection of cultured plant cells with *Agrobacterium tumefaciens*, a plant pathogen with the inherent ability to transfer a particular DNA segment into the nucleus of the host cell allowing its transcription. The inherent ability of *Agrobacterium tumefaciens* to infect plant cells has allowed it to be used as a vector for genetic manipulation (De la Riva *et al.*, 1998; Gelvin, 1998; Hansen and Wright, 1999; Gelvin, 2003). The point of insertion of the transgene into the genome of a plant cell is random and each insertion is referred to as an “event” (Nester, 2008; Lievens *et al.*, 2015).

Genetic engineering is extensively utilized in agriculture, to produce crop varieties with improved agricultural traits (König *et al.*, 2004). The major commercial genetically modified (GM) crop traits include insect resistance and herbicide tolerance (James, 2015). Insect resistant plants are modified to produce an endotoxin that kills insect pests. Herbicide tolerant (HT) plants are engineered to be tolerant to the application of herbicides during the growing season to control weeds. Crops have also been modified for other traits, including disease resistance, drought tolerance and improved nutritional content (Gasser and Fraley, 1989; Uzogara, 2000; Nester, 2008; Lievens *et al.*, 2015). The application of GM crops is considered to have resulted in lower use of pesticides, labour, machinery and fuel (Gouse, 2014). In general, GM crops are considered to have made a positive contribution to agriculture since its introduction in 1996, by reducing insect damage and improving crop management (Qaim, 2010).

1.2 GM crop production worldwide

GM crops were first commercialised in 1996 in the United States of America (USA) (Mannion and Morse, 2013). By 2014, 28 countries planted more than 181 million hectares (ha) of GM crops (James, 2014). Currently there are four major GM crops produced commercially: canola, cotton, maize and soybean. Other GM crops include alfalfa, papaya, potato, squash, sugar beet and sweet peppers (James, 2013). The ten major GM crop producing countries include the USA (producing 73.1 million ha of GM crop), Brazil (producing 42.2 million ha of GM crop), Argentina (producing 24.3 million ha of GM crop), India (producing 11.6 million ha of GM crop), Canada (producing 11.6 million ha of GM crop), China (producing 3.9 million ha of GM crop), Paraguay (producing 3.9 million ha of GM crop), Pakistan (producing 2.9 million ha of GM crop), South Africa (producing 2.3 million ha of GM crop) and Uruguay (producing 1.6 million ha of GM crop) (James, 2015).

GM crops have been commercially planted in South Africa since 1998 (Du Plessis, 2003; SA DAFF, 2005; SANBI, 2010). In 2015, GM crop production in South Africa amounted to 2.3 million ha making it the ninth biggest GM crop producing country in

the world (James, 2015). It is estimated that 90% of maize (1.8 million ha), 95% of soybean (508,000 ha) and 100% of cotton (12,000 ha) produced in South Africa is GM (James, 2015). The GM traits approved in South Africa for maize include herbicide tolerance (comprising 15.8% or 284,000 ha of GM maize), insect resistance (comprising 30.5% or 550,000 ha of GM maize) and stacked events containing both traits (herbicide tolerance and insect resistance) (comprising 53.4% or 940,000 ha of GM maize) (James, 2015). The only GM trait approved in South Africa for soybean is herbicide tolerance (comprising 100% or 508,000 ha of GM soybean) and for cotton the only approved trait is insect resistance (comprising 100% or 12,000 ha of GM cotton) (James, 2015; SA DAFF, 2015). In South Africa, herbicide tolerance is the major trait in approximately 75% of the GM crops planted. There are currently two HT events approved for maize in South Africa namely GA21 and NK603 and one for soybean, namely GTS40-3-2 (SA DAFF, 2015).

HT crops have the ability to tolerate specific broad-spectrum herbicides including glyphosate, glufosinate and 2,4-dichlorophenoxyacetic acid (2,4D) (Benbrook, 2016). HT crops allow the direct application of herbicide to eliminate weeds during the growing season without causing crop damage (Madsen and Streibig, 2003). Glyphosate is the major herbicide used on HT crops worldwide including South Africa (Bonny, 2016).

1.3 Glyphosate use in agriculture

Glyphosate is approved in more than 130 countries and is considered the most widely used herbicide in the world (Dill *et al.*, 2010). Since the introduction of GM HT crops in 1996, global use of glyphosate has increased, with a 10 fold increase recorded in 2012 amounting to approximately 720,000 tonnes compared to only 67,078 tonnes in 1995 (Hilton, 2012; Benbrook, 2016). In 2012, South Africa used approximately 40,775 tonnes of glyphosate, mostly in the application on HT crops (Gouse, 2014).

Glyphosate is a broad-spectrum, non-selective, systematic herbicide used to kill weeds (Duke *et al.*, 2003; Dill *et al.*, 2010). When applied at lower concentrations, glyphosate is also used as a desiccant (Duke *et al.*, 2003; IARC, 2015). Crop

desiccation using glyphosate is common practise in the USA. However, the extent of using glyphosate as a desiccant in South Africa is not known. Glyphosate is generally applied by means of directed spray application (USA EPA, 1993). Upon application, glyphosate is absorbed by the foliage and distributed throughout the entire plant (Duke *et al.*, 2003; Arregui *et al.*, 2004). Glyphosate disrupts the shikimate pathway by inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), involved in the synthesis of the essential amino acids phenylalanine, tyrosine and tryptophan, which is critical for plant growth (Padgett *et al.*, 1995; Funke *et al.*, 2006). The inhibition of the EPSPS enzyme results in plant death within a matter of days (Franz *et al.*, 1997). HT crops are genetically engineered to express a glyphosate insensitive EPSPS enzyme allowing direct application of glyphosate to selectively kill weeds without crop damage (Dill *et al.*, 2010).

It is estimated that approximately 45% of glyphosate produced worldwide is used in the production of HT crops (Dill *et al.*, 2010). However, the continuous application of glyphosate on HT crops has led to the emergence of resistant weeds (Benbrook, 2012). Currently, approximately 31 weed species worldwide have developed resistance to glyphosate (Reinhardt, 2012). As a result of this, glyphosate is being applied at higher concentrations to combat weed resistance (Benbrook, 2012). Recently, Benbrook (2016) reported that the amount of glyphosate applied to HT soybean in the USA increased from 0.7 kg/ha in 1996 to approximately 1.1 kg/ha in 2014. Benbrook (2016) suggested that the upward trend in glyphosate use will likely continue, increasing the glyphosate levels present in the environment and potentially increasing animal and human exposure to the herbicide.

1.4 Glyphosate tolerance levels

The maximum residue level (MRL) is the maximum concentration of pesticide residue legally allowed in food or animal feed based on “good” agricultural practice (EFSA, 2009; EFSA, 2015, Codex Alimentarius, 2015). The main purpose of the MRL is to ensure fair practice in international food trade (FAO, 2013). MRLs are used as a regulatory standard to help monitor whether a pesticide is applied as approved. Pesticide residue in food or feed exceeding the MRL indicates misuse of a chemical

during agricultural application. The MRL, although not considered a safety standard has a direct influence on the amount of pesticide residue present in the food chain. The MRL for a pesticide is established at a level that ensures that the residue levels in food do not exceed the acceptable daily intake (ADI) for the particular pesticide in a country (FAO, 2013).

The MRLs are determined by individual countries, as well as by Codex Alimentarius of the Food and Agriculture Organisation of the United Nations (FAO) and WHO (Codex Alimentarius, 2015). Countries which do not have established MRLs for pesticides may use the MRLs as established by Codex Alimentarius (Table 1.1) (FAO, 2013; Codex Alimentarius, 2015). A number of factors are taken into consideration when the MRL are set for a pesticide such as glyphosate in commodities (FAO, 2006). These factors include the minimum effective dose, the standard application dose rate, the time between harvest and consumption and the climatic conditions affecting pesticide efficacy (FAO, 2006). As a result of this, the MRL for a particular pesticide may differ for different commodities. For example, for a crop like bananas, where glyphosate is unlikely to be used either in weed management or as a desiccant, the MRL is 0.05 mg/kg (Table 1.1) (FAO, 2013). However, for crops like wheat, hay or alfalfa where glyphosate is likely to be used as a desiccant or for weed control, the MRL range from 300 mg/kg to 500 mg/kg (Table 1.1) (FAO, 2013). The South African Department of Agriculture, Forestry and Fisheries (SA DAFF) has established glyphosate MRLs for all applicable commodities in South Africa (Table 1.1) (SA DAFF, 2016). The MRLs established for crops in South Africa is similar to those established by Codex Alimentarius, the European Union (EU) and the USA.

Table 1.1: Maximum residue levels (mg/kg) established for glyphosate. MRLs for glyphosate as established by Codex Alimentarius (Codex), the United States of America Environmental Protection Agency (USA EPA), the European Food Safety Authority (EFSA) and the South African Department of Agriculture, Forestry and Fisheries (SA DAFF).

Commodities	Codex¹ (mg/kg)	USA EPA² (mg/kg)	EFSA³ (mg/kg)	SA DAFF⁴ (mg/kg)
Banana	0.05	0.2	0.1	0.1
Beans (dry)	2	5	2	2
Sugar cane	2	2	0.1	0.5
Lentil (dry)	5	8	10	NA
Peas (dry)	5	8	10	10
Maize	5	5	1	2
Sunflower seed	7	85	20	20
Soybean (dry)	40	20	20	20
Rape seed	20	20	10	20
Wheat bran, Unprocessed	20	30	10	30
Wheat fodder (dry)	300	NA	NA	NA
Hay of grasses (dry)	500	NA	NA	NA
Alfalfa fodder (dry)	500	NA	NA	NA

1 Codex Alimentarius (www.fao.org/docrep/009/a0209e/a0209e0d.htm)

2 USA EPA (<http://www.epa.gov/opp00001/reregistration/REDS/factheets/0178fact.pdf>)

3 EFSA (<http://www.efsa.europa.eu/en/topics/topic/pesticides.htm>)

4 SA DAFF ([http://www.SA DAFF.gov.za/SA DAFFweb3/Branches/Agricultural-Production-Health-Food-Safety/Food-Safety- Quality-Assurance/Maximum-Residue-Limits](http://www.SA%20DAFF.gov.za/SA%20DAFFweb3/Branches/Agricultural-Production-Health-Food-Safety/Food-Safety-Quality-Assurance/Maximum-Residue-Limits))

Not applicable (NA): No values have been established for particular crops in these countries

Since the introduction of GM HT crops regulatory authorities have frequently changed the MRLs for glyphosate as a result of increased application. In 1999, the glyphosate MRL for soybean was raised from 0.1 mg/kg to 20 mg/kg in the USA and Europe. Likewise in 2004, the glyphosate MRL for soybean was raised from 0.2 mg/kg to 10 mg/kg in Brazil (Bøhn *et al.*, 2014). Bøhn *et al.* (2014) suggested that the MRL adjustments were made in response to actual observed increases in the glyphosate residue detected in GM HT soybean. It has been suggested that the recurrent planting of HT crops and glyphosate application on the same fields without crop rotation or the use of different herbicides has contributed to the emergence of resistant weeds. In an effort to combat weed resistance, higher concentrations of glyphosate are applied to HT crops. The increase in the amount of glyphosate sprayed on HT crops has subsequently resulted in an increase in levels of glyphosate residue in HT grains as well as the environment (Benbrook, 2016).

1.5 Safety assessment of glyphosate

The commercial formulation of glyphosate contains surfactants which enhance its herbicidal properties. These surfactants facilitate absorption and increase the degree of rainfastness of the herbicide and ensure that it is not washed off by rain or during irrigation (Duke *et al.*, 2003). The United States of America Environmental Protection Agency (USA EPA) does not require safety testing of the surfactants used in pesticides, since they are considered to have no pesticidal properties (Herzfeld and Sargent, 2012). As a result of this, there are no standards on the composition and safety of the non-pesticidal ingredients of pesticide formulations worldwide (Herzfeld and Sargent, 2012). Several studies, reports and reviews on the safety of pure glyphosate have concluded that it is safe for humans if applied at the correct agricultural concentration (Williams *et al.*, 2000). The acute toxicity of glyphosate and its major metabolite aminomethylphosphonic acid (AMPA) has been tested in animal feeding trials and no adverse effects have been found (Williams *et al.*, 2000; Williams *et al.*, 2012). Furthermore, in 1993, the USA EPA classified both glyphosate and AMPA in Category E, which is described as “Evidence of Non-carcinogenicity”, based on the lack of convincing evidence of carcinogenicity in numerous studies (USA EPA, 1993). In 1994, the WHO reaffirmed the findings that there was no evidence that

glyphosate and AMPA were harmful to humans and that both glyphosate and AMPA had negligible levels of acute toxicity (WHO, 1994).

In contrast to studies on pure glyphosate, several studies on glyphosate in formulation (glyphosate with surfactants) have reported toxicity and carcinogenicity. A study by Gasnier *et al.* (2009) demonstrated that glyphosate in formulation at 5 ppm (5 mg/kg) had toxic effects and resulted in cell death of human liver, umbilical cord and placental cells within 24 hours of exposure. Furthermore, Gasnier *et al.* (2009) also indicated that glyphosate in formulation at 0.5 ppm (0.5 mg/kg) caused endocrine disruption in human liver cells within 24 hours of exposure (Table 1.3). A more recent study by Belle *et al.* (2012) determined that 8 mM (1,300 mg/kg) of glyphosate in formulation inhibited the cell replication of human embryonic cells within 24 hours of exposure (Table 1.3). Belle *et al.* (2012) concluded that the concentration of glyphosate in formulation used in their study was far below the prescribed concentration of 40 mM recommended for herbicide application during agricultural practice. A study by Koller *et al.* (2012) found that glyphosate in formulation at 10 mg/L to 20 mg/L (5.7 mg/kg to 11.4 mg/kg), caused DNA damage and at 40 mg/L (22 mg/kg) caused membrane damage and mitochondrial impairment in human epithelial cells (Table 1.3). A study by Young *et al.* (2015) demonstrated that glyphosate in formulation was cytotoxic to human placenta cells at concentrations ranging from 0.005 mM to 0.008 mM (0.85 mg/kg to 1.35 mg/kg) (Table 1.3). Young *et al.* (2015) confirmed that the surfactants within the glyphosate formulation had a major effect on the toxicity of the herbicide and demonstrated that glyphosate in formulation exhibited similar toxicity at a concentration of 2000 times lower than pure glyphosate. Furthermore, Belle *et al.* (2012) suggested that glyphosate on its own should not be considered a herbicide, since without surfactants it is not permeable and cannot be absorbed by plant cells. Williams *et al.* (2012) argued that safety studies on glyphosate in formulation are irrelevant since glyphosate toxicity is as a result of “surfactants present in the formulation” and not due to glyphosate itself. Viljoen (2013) suggested that the argument of Williams *et al.* (2012) was “irrelevant, since it is the formulation that is being applied to the plant in practice and it is part of the herbicide complex of chemicals taken up by the plant”.

In 2015, the International Agency for Research on Cancer (IARC) assessed the carcinogenicity of several pesticides, including glyphosate. The IARC changed its classification of glyphosate from “possibly carcinogenic” to “probably carcinogenic” to humans (Table 1.2) (IARC, 2015). The IARC evaluated approximately 260 documents, reports and studies and re-classified glyphosate as a probable human carcinogen based on: limited evidence of carcinogenicity in humans, sufficient evidence of carcinogenicity in experimental animals and strong evidence of genotoxicity and oxidative stress in both human and animal cells (IARC, 2015). The IARC report concluded that glyphosate causes DNA and chromosomal damage in mammalian and non-mammalian cells at low concentrations (IARC, 2015).

Table 1.2: IARC classification system for pesticides.

IARC classification system	Description
Group 1	Carcinogenic to humans
Group 2A	Probably carcinogenic to humans
Group 2B	Possibly carcinogenic to humans
Group 3	Not classifiable in terms of human carcinogenicity
Group 4	Probably not carcinogenic to humans

IARC/WHO (<http://www.iarc/who.monographs.iarc.fr/ENG/Classification/>)

The IARC report listed approximately 109 studies indicating DNA and chromosomal damage in mammalian and non-mammalian cells as a result of glyphosate in formulation. A study by Alvarez-Moya *et al.* (2014) reported that glyphosate in formulation at a concentration of 0.12 mg/L (0.069 mg/kg) caused DNA damage in human lymphocytes (Table 1.3). A further study by Roustan *et al.* (2014) indicated that glyphosate in formulation induced chromosomal breakage in hamster ovary cells at a concentration of only 0.01 mg/L (0.006 mg/kg) (Table 1.3). Similar results were found in fish by Moreno *et al.* (2014) indicating that glyphosate in formulations caused

DNA strand breaks in liver and gill cells at a concentration of 0.058 mg/L (0.033 mg/kg) (Table 1.3). DNA and chromosomal damage leads to an increased mutation rate and subsequently an increased risk for developing cancer. The only cancer in humans with a significant link to glyphosate is Non-Hodgkins lymphoma (IARC, 2015). Case-control studies from the USA and Sweden reported a statistically significant increased risk for Non-Hodgkins lymphoma associated with glyphosate exposure (Hardell *et al.*, 2002; De Roos *et al.*, 2003; Eriksson *et al.*, 2008; Orsi *et al.*, 2009). Animal studies have indicated that continuous exposure to glyphosate resulted in a significant increase in the risk for pancreatic islet cell adenoma, renal tubule carcinoma, hepatocellular adenoma and thyroid C cell adenoma in male and female mice (USA EPA, 1986; USA EPA, 1991). Thus as a result, the IARC has concluded that there is limited evidence of carcinogenicity in humans and convincing evidence of carcinogenicity in animals as a result of exposure to glyphosate.

The IARC report has been widely criticised. Agricultural companies have claimed that the IARC misinterpreted or incorrectly weighed some of the data it reviewed before classifying glyphosate as a “probable human carcinogen” (Plume, 2015). In April 2016, a group of 16 scientists reviewed the IARC report and produced a detailed critique of the IARC report on glyphosate (Williams *et al.*, 2016). Williams *et al.* (2016) indicated that their analysis of existing data did not support the IARC’s conclusion that glyphosate is a “probable human carcinogen”. Furthermore, Williams *et al.* (2016) stated that their review was consistent with previous regulatory assessments and concluded that “glyphosate is unlikely to pose a carcinogenic risk to humans”. In the “declaration of interest” in Williams *et al.* (2016), 12 of the 16 authors previously served as consultants, or worked for, the companies producing glyphosate. The critique of the IARC report was also funded by a major glyphosate manufacturer.

Since the release of the IARC report several regulatory agencies have reviewed the safety of glyphosate. In 2015, the Pest Management Regulatory Agency (PMRA) stated that there was no evidence that glyphosate posed a health risk at the prescribed agricultural dose. Similarly, the European Food Safety Authority (EFSA) re-evaluated the health risk of glyphosate and concluded that “glyphosate is unlikely to pose a carcinogenic hazard to humans and the evidence does not support classification with regards to its carcinogenic potential” (EFSA, 2015). In May 2016, a summary report

issued after a joint meeting by the FAO and WHO on pesticide residue concluded that glyphosate is unlikely to pose carcinogenic risk to humans through dietary exposure (JMPR, 2016). It should be noted that the regulatory agencies as well as Williams *et al.* (2016) used an overall weight of evidence approach to reach conclusions regarding the genotoxicity of glyphosate. Taking into consideration that several studies on glyphosate are industry funded and that the majority of published research on glyphosate safety is based on pure glyphosate and not on glyphosate in formulation, the validity of an overall weight of evidence approach is questionable.

Recently, Portier *et al.* (2016) comprising a group of 94 scientists, reviewed the EFSA evaluation of the IARC report on the safety of glyphosate and concluded that there were serious flaws in the EFSA report. Portier *et al.* (2016) suggested that EFSA dismissed any association of glyphosate with cancer, without clear explanation or justification and ignored important evidence of genotoxicity. Furthermore, Portier *et al.* (2016) found it problematic that EFSA based their evaluation on the Renewal Assessment Report (RaR) giving almost no weight to published literature while relying heavily on studies provided by the pesticide industry and not available in the public domain. Portier *et al.* (2016) criticised EFSA and other regulatory bodies that concluded that glyphosate was safe. Furthermore, Portier *et al.* (2016) suggested that regulatory authorities are under pressure to conclude that glyphosate is safe since it is the major pesticide used in agriculture worldwide. Portier *et al.* (2016) concluded that the re-classification of glyphosate as a probable carcinogen by the IARC working group accurately reflected the current results of published scientific literature on glyphosate. Considering the extreme viewpoints on glyphosate safety, it is apparent that more research is required on the safety of this herbicide.

Table 1.3: Summary of studies indicating the toxicity of glyphosate in formulation

Study	Tissue studied	Minimum concentration of glyphosate	Observation
Gasnier <i>et al.</i> , (2009)	Human liver cells Umbilical cord cells Placental cells	5 mg/kg	Cell death
	Human liver cells	0.5 mg/kg	Endocrine disruption
Belle <i>et al.</i> , (2012)	Human embryonic cells	1,300 mg/kg	Inhibition of cell replication
Koller <i>et al.</i> , (2012)	Human epithelial cells	22 mg/kg	Membrane damage Mitochondrial impairment
	Human epithelial cells	5.7 to 11.4 mg/kg	DNA damage
Young <i>et al.</i> , (2015)	Human placental cells	0.85 to 1.3 mg/kg	Cytotoxicity
Alvarez-Moya <i>et al.</i> , (2014)	Human lymphocytes	0.069 mg/kg	DNA damage
Roustan <i>et al.</i> , (2014)	Hamster ovary cell	0.006 mg/kg	Chromosomal breakage
Moreno <i>et al.</i> , (2014)	Fish liver cells Fish gill cells	0.033 mg/kg	DNA strand breaks

1.6 Detection of glyphosate as a result of agricultural application

1.6.1 Detection of glyphosate in HT maize and HT soybean

Glyphosate in formulation is applied to GM HT crops one to three times during the growing season to control weeds (Krüger *et al.*, 2014a). After application, glyphosate is absorbed and distributed to all parts of the HT plant tissue (Duke *et al.*, 2003; Robinson, 2009). When glyphosate was initially applied in agriculture it was not known that it would later be detected in the grain of HT crops. Published data on glyphosate residue in HT crops is sparse (Benbrook, 2016). However, studies have detected glyphosate in HT maize from the USA as well as HT soybean from the USA and Argentina (Reddy *et al.*, 2004).

The first study detecting glyphosate residue in HT soybean was by Duke *et al.* (2003). Results from the study indicated that glyphosate was present in the foliage of HT soybean at levels reaching 3.08 mg/kg. A more recent study by Bøhn *et al.* (2014) tested commercially grown dry soybean samples in the USA and detected glyphosate in all HT soybean samples, at levels reaching up to 3.3 mg/kg. Then (2013) tested 11 HT soybean samples from Argentina and detected glyphosate at levels of up to 26 mg/kg. Results from Then (2013) indicated that some samples contained glyphosate at levels exceeding the MRLs established for soybean in Argentina (20 mg/kg). Then (2013) suggested that this was as a result of glyphosate being applied at concentrations much higher than recommended, most likely due to increasing weed resistance. Furthermore, Then (2013) concluded that the high residue levels within the HT soybean may have a serious health impact through food consumption and suggested that the MRLs for glyphosate in food should be reduced.

In 2005, the FAO summarized the findings from 78 trials done on HT maize produced within the USA. Results indicated that glyphosate in maize fodder was detected at 92 mg/kg which was expected due to higher concentrations being sprayed to desiccate the crop. Compared to this, only 2.2 mg/kg of glyphosate was detected in maize grain (FAO, 2005). Overall, the glyphosate levels in HT maize are considered low with all studies detecting glyphosate at below the established MRL for maize in the USA (5 mg/kg).

In some plant species such as HT soybean, glyphosate is metabolised to its primary metabolite AMPA. In HT maize, no AMPA has been detected, suggesting that glyphosate is not further metabolised in maize (Reddy *et al.*, 2004; Gomes *et al.*, 2014). AMPA is an active metabolite of glyphosate and exhibits phytotoxic ability by disrupting the chlorophyll biosynthesis within plant cells. Then (2013) suggested that since AMPA was an active metabolite of glyphosate, the sum of glyphosate and AMPA should be calculated to determine the residue level per sample. Duke *et al.* (2003) detected AMPA in HT soybean at levels reaching up to 25 mg/kg. If this value is combined with the glyphosate level detected by Duke *et al.* (2003) (3.08 mg/kg), the total residue in HT soybean would reach approximately 28.08 mg/kg, exceeding the MRL established for soybean in the USA (20 mg/kg). Similarly, Then (2013) detected AMPA at levels of up to 47 mg/kg in HT soybean from Argentina. The total glyphosate and AMPA levels detected for some HT soybean samples, reached up to 97.4 mg/kg and five of the 11 samples tested contained residue levels exceeding the MRL established for soybean in Argentina (20 mg/kg). These findings suggest that glyphosate is applied at higher rates than what is indicated by the pesticide producer as a result of weed resistance. In South Africa, weed resistance to glyphosate has not been reported extensively and data on this is sparse. As a result, it is not known if weed resistance has influenced the rate of glyphosate application in South Africa.

1.6.2 Glyphosate in processed food products and water

Due to its frequent use in agriculture, glyphosate residue is present in various food products as well as in water. Various countries including South Africa have established routine monitoring of pesticides in food. However, this is mostly aimed at fresh fruit, vegetables and grain (Swanepoel, 2014). Limited research has been conducted on the presence of glyphosate in processed foods. Nonetheless, recent studies have detected glyphosate in various processed food products (McQueen *et al.*, 2012; Swanepoel, 2014; Rubio *et al.*, 2014). In 2014, 15.4% (30 out of 195) of bread samples from the United Kingdom (UK) were found to contain glyphosate at concentrations of up to 0.100 mg/kg (PRIF, 2015). Similarly, a study by Swanepoel (2014) in South Africa confirmed that glyphosate was present in 88.0% (seven out of

eight) of white bread samples tested but did not specify the concentrations of glyphosate detected. Rubio *et al.* (2014) also detected glyphosate in 60.0% of honey and 36.0% soy sauce products tested from the USA, with concentrations of up to 0.564 mg/kg detected. In a report by EFSA (2009) it was stated that glyphosate cannot be removed from food by washing, processing or cooking. Although the level of glyphosate detected in studies on processed food products were lower than the established MRLs for food commodities, the MRL should not be considered a safety standard but rather a guideline for “good” agricultural practice.

Several studies have confirmed that glyphosate is detected in water in several countries. A report by the WHO (2005) found that glyphosate was present in ground water samples at levels of up to 5.15 mg/L (2.94 mg/kg) and 1.7 mg/L (0.97 mg/kg) in Canada and the USA, respectively. More recently, Battaglin *et al.* (2014) tested more than 3,700 water samples, sourced from 38 states in the USA and indicated that glyphosate was detectable in 53.0% of the water samples, at concentrations of up to 0.470 mg/L (0.269 mg/kg). However, Battaglin *et al.* (2014) concluded that the glyphosate levels detected in the water were considered low as they were below the maximum contaminant level established for drinking water in the USA (0.7 mg/L) (USA EPA, 2015).

The glyphosate levels detected in food and water are considered safe as it is below the MRL (FAO, 2013). However, it is surprising that the tolerated residual levels for glyphosate differ so greatly for different crops and water. For water, the maximum contaminant level of glyphosate is 0.7 mg/L (0.4 mg/kg) (USA EPA, 2015), yet for food products like soybean and maize much higher MRLs (up to 40 mg/kg for soybean and 5 mg/kg for maize) are considered safe in the USA. Both food and water are essential for human survival and are consumed daily. It is questionable why the glyphosate in water is limited to 0.7 mg/L while in crops concentrations of a more than 10 fold are tolerated. This demonstrates the inconsistency when the MRLs for commodities and maximum contaminant level for water are established.

There is currently no published data on the levels of glyphosate in HT maize and HT soybean in South Africa. Since HT maize and HT soybean are extensively used in agriculture in South Africa, it is expected that glyphosate is likely to be present in the

grain. Furthermore, considering that glyphosate cannot be removed from grain by cooking, washing or processing it is likely that glyphosate would be present in food and feed. There is also no data for the levels of glyphosate in water in South Africa.

1.6.3 Glyphosate in animal tissue and excretions

Several studies have tested the fate of glyphosate in animals including humans. Research on laboratory animals has confirmed the absorption of glyphosate within the gastrointestinal tract after being fed glyphosate treated feed. Animal feeding studies have also confirmed the distribution of absorbed glyphosate to the tissue of all major organs including the small intestine, kidneys, liver, heart, lungs, blood and bone (Brewster *et al.*, 1991; Krüger *et al.*, 2014a).

A study by Brewster *et al.* (1991) suggested that approximately 35% to 40% of pure glyphosate, administered to Sprague-Dawley rats via oral intubation, was absorbed in the gastrointestinal tract. Brewster *et al.* (1991) also confirmed the distribution of absorbed glyphosate within the body and detected peak glyphosate levels in the small intestine, kidneys, liver, blood and bone within six hours after application. While Brewster *et al.* (1991) did not report on the amount of glyphosate detected in each tissue, they did state that the glyphosate levels in all tissues declined rapidly. Furthermore, they suggested that urine and faeces were important routes for glyphosate excretion (Brewster *et al.*, 1991). A more recent study by Krüger *et al.* (2014a) also reported the presence of glyphosate in the intestine, liver, muscle, spleen, kidneys and urine of dairy cows and fattening rabbits in Germany. The animals were fed feeds including soy, corn and other grains, treated with different concentrations of glyphosate post harvesting (Krüger *et al.*, 2014a). However, the study did not determine the amount of glyphosate absorbed or the extent of glyphosate excretion. Krüger *et al.* (2014b) also investigated the reason for the severe malformation in piglets on a Danish pig farm and suggested that glyphosate may be a contributing factor. Tissue samples from piglets born with severe malformations were tested and glyphosate was detected in all samples. Kruger *et al.* (2014b) concluded that ingested glyphosate is transferred across the placental barrier during sow

pregnancy, but that further studies are necessary to confirm or exclude the role of glyphosate in piglet birth malformation.

Although not proven, the possibility of glyphosate bio-accumulation within animal tissue cannot be ignored. It has been suggested that ingested glyphosate is rapidly excreted from the body by means of urine and faeces (Brewster *et al.*, 1991). However, it is not known whether the glyphosate absorbed in organ tissue follows the same excretion rate. Furthermore, the detection of glyphosate in animal tissue as a result of being fed glyphosate treated feed indicates that it is transferred within the food chain.

Studies on the fate of glyphosate in humans are limited to urine testing. Studies have confirmed that glyphosate is detectable in the urine of humans from farming and urban communities in the USA and Europe. One of the earliest studies testing for glyphosate in human urine was done by Acquavella *et al.* (2004). They tested urine samples of 127 individuals from farms in the USA and reported that sixty percent had detectable levels of glyphosate with concentrations of up to 233 µg/L (0.133 mg/kg) (Acquavella *et al.*, 2004). It was suggested that the glyphosate in the urine samples was as a result of occupational exposure during agricultural application (Acquavella *et al.*, 2004). A similar study by Curwin *et al.* (2007) analysed the urine samples of individuals from farming communities in USA and as a control group, used urine samples of individuals from non-farming communities. Curwin *et al.* (2007) reported that glyphosate was detected in the majority of samples (60% of adults and 80% of children) with concentrations of up to 18 µg/L (0.010 mg/kg) and that there was no significant difference in urinary glyphosate concentration between individuals from farming or non-farming communities. The findings of Curwin *et al.* (2007) suggest that other sources of glyphosate exposure should be considered as non-farming individuals are not exposed to glyphosate as a result of occupational application. Several studies have confirmed the presence of glyphosate in grain (FAO, 2005, Then, 2013, Bøhn *et al.*, 2014), processed food (Rubio *et al.*, 2014; Swanepoel, 2014), animal tissue (Krüger *et al.*, 2014 a/b) and water (Battaglin *et al.*, 2014) which all contribute substantially to the human diet. These findings suggest that diet may contribute a greater role in exposing individuals to glyphosate than initially thought. Furthermore, considering the results from animal studies detecting glyphosate in the tissue of all

major organs after being fed glyphosate treated feed (Brewster *et al.*, 1991; Krüger *et al.*, 2014a), it can be argued that a similar result may be expected in human tissue. With the recent re-classification of glyphosate as a “probable human carcinogen”, residue within the human body, whether from occupational exposure or dietary intake, is concerning and needs further investigation.

1.7 Conclusion

GM crops were introduced for commercial planting in 1996 and are considered to have made a positive contribution to crop management in agriculture over the last two decades (Qaim, 2010). Herbicide tolerance is the predominant trait in all GM crops and accounts for approximately 85% of GM crop production worldwide (James, 2015). South Africa is a major GM crop producing country and 75% of GM crops are herbicide tolerant (James, 2015). The most widely used herbicide in the world is glyphosate, which is also used on GM HT crops (Bonny, 2016).

In recent years, several studies have confirmed the absorption of glyphosate by GM HT plants, including maize and soybean, and its distribution to all parts of the plant tissue including the grain (Duke *et al.*, 2003; Robinson, 2009; Bøhn *et al.*, 2014). When GM HT crops were initially commercialized it was not known that glyphosate would be detectable in the grain of the crops treated with the herbicide. The levels of glyphosate detected in the grain of HT maize and HT soybean is generally below the MRLs established for these commodities. However, several recent studies have shown that glyphosate in formulation exhibits genotoxic effects at concentrations similar to what has been detected in HT grain (Alvarez-Moya *et al.*, 2014; Roustan *et al.*, 2014; Young *et al.*, 2015).

Several animal feeding studies have confirmed that glyphosate is absorbed and can be detected in all major organs of animals after exposure to glyphosate treated feed (Brewster *et al.*, 1991; Krüger *et al.*, 2014a). Brewster *et al.* (1991) estimated that approximately 30% to 40% of ingested glyphosate is absorbed in the gastrointestinal tract and is rapidly excreted from the body by means of urine and faeces (Brewster *et al.*, 1991). However, it is not known whether the glyphosate absorbed in organ tissue

follows the same excretion rate and research is needed to determine whether there is any bio-accumulation of glyphosate in animal tissue.

Several studies have detected glyphosate in human urine (Acquavella *et al.*, 2004; Curwin *et al.*, 2007). Since these studies focused mostly on farming communities, it was suggested that the glyphosate levels present in urine was as a result of occupational exposure (Acquavella *et al.*, 2004). However, a recent study investigating the urinary glyphosate concentrations of farming and non-farming households concluded that glyphosate was detected in the urine of both groups at similar concentrations. Furthermore, a limited number of studies have also detected glyphosate at low levels in processed food products as well as in water (Battaglin *et al.*, 2014; Rubio *et al.*, 2014; Swanepoel, 2014). The latter results suggest that there may be other sources of glyphosate exposure either through diet or water intake.

In South Africa, maize (in the form of maize meal) is a major staple and soybean an important source of protein. The majority of GM maize (68%) and soybean (100%) grown in South Africa is HT (James, 2015). It is currently not known to what extent glyphosate may be present in the South African food chain, specifically regarding maize and soybean containing food products. Considering the potential uncertainty regarding the safety of glyphosate, it is important to clarify the extent of the presence of glyphosate in South African food products.

CHAPTER 2: RESEARCH AIM AND METHODOLOGY

2.1 Rationale

Maize is a major staple and soybean an important source of protein in South Africa. It is estimated that 61% of maize and 95% of soybean produced in South Africa is HT (James, 2015). The herbicide most widely used to treat HT crops is glyphosate. Studies have shown that after application, glyphosate is absorbed by HT plants and distributed to all plant tissue including grain (Duke *et al.*, 2003; Then, 2013). Animal studies have demonstrated that glyphosate is absorbed from feed and detected in the tissue of all major organs (Brewster *et al.*, 1991; Krüger *et al.*, 2014a; Krüger *et al.*, 2014b). However, up until 2015, glyphosate was considered safe for humans and the environment. In 2015, the IARC changed its classification of glyphosate from “possibly carcinogenic” to “probably carcinogenic” to humans. The IARC based the re-classification of glyphosate on accumulating evidence showing the potential genotoxic properties of glyphosate (IARC, 2015). It is estimated that approximately 500 g of cooked maize meal is consumed per person daily in poor households in South Africa (Payne, 2011). In addition to this, soybean is an important source of protein and is added to various food products to increase the protein content. It is currently not known to what extent glyphosate is present in food products in South Africa, of which maize or soybean are the primary constituent. Taking into consideration that glyphosate may be potentially carcinogenic and is not removed from food by washing, cooking or processing (EFSA, 2009), it is important to know to what extent glyphosate is present in food products in South Africa.

2.2 Aim of Study

The primary aim of this study was to determine whether glyphosate is present in commercially available food products in South Africa that contain maize or soybean as the major constituent. The secondary aim was to detect and quantify the presence of GM HT events in maize (NK603 and GA21) and soybean (GTS40-3-2) food products. A minor aim was to use the available data to evaluate the maize and

soybean food products in terms of compliance with the South African Consumer Protection Act (SACPA) regarding GM labelling.

2.3 Study Design

This study was performed as an analytical experimental study. Commercially available food products which contained maize or soybean as a primary constituent were identified and purchased from retail stores in South Africa. The enzyme-linked immunosorbent assay (ELISA) was used to test all products for the presence and levels of glyphosate. Furthermore, event specific Real-time polymerase chain reaction (PCR) screening was used to determine whether the products contained GM HT events approved in South Africa for maize and soybean. Food products positive for one or more of the GM HT events were quantified using Real-time PCR in order to determine the percentage GM HT event present in each product. Finally, the data from HT event quantification was used to evaluate whether the products tested were compliant with the South African Consumer Protection Act (2008) in terms of GM labelling.

2.4 Product selection and sampling

A total of 81 food products were selected from retail outlets including Pick 'n Pay, Shoprite, Checkers, Spar, Dischem and Woolworths according to product availability during 2015. Products were selected to include as many different product brands as possible (Table 2.1). During sampling, only products which contained maize and/or soybean as the major constituent in raw or processed form were selected. Products were arranged into three categories using their ingredients list as a guideline: samples containing maize, samples containing soybean and samples containing both maize and soybean as a primary constituent. The texturized soy protein products and corn-soy blends listed both maize and soybean as a primary constituent and were tested for both HT maize and soybean events. For soybean, infant milk and soy flour, only one brand was commercially available.

Table 2.1: The food products selected for this study.

Sample category	Number of products
Maize products	
Maize meal	20
Instant maize meal	2
Beer powder	2
Maize grits	5
Maize rice	3
Polenta	5
Corn flakes	7
Corn chips	10
Maize pasta	3
Total	57
Soybean products	
Soybeans	1
Soy milk	8
Infants milk	1
Soy flour	1
Total	11
Texturized soybean protein products	
Texturized soy protein (<i>containing only soybean</i>)	4
Texturized soy protein (<i>containing maize and soybean</i>)	3
Total	7
Corn-soy products	
Corn-soy blend ¹	6
Total	6

¹ Corn-soy blends are precooked cereals containing milled maize and soybean

2.4.1 Inclusion criteria

Food products in which maize and/or soybean was the primary constituent of the products based on the ingredient list.

2.4.2 Exclusion criteria

Food products in which maize and/or soybean was not the primary constituent of the products based on the ingredient list.

2.5 Methodology for glyphosate screening

2.5.1 Sample preparation and glyphosate determination

Glyphosate was detected and quantified using an ELISA kit according to the manufacturer's instructions (Abraxis, USA) (Rubio *et al.*, 2003). Samples were homogenized where necessary, using a food blender to a maximum particle size of 2.5 mm². Sample preparation included the addition of 10 mL of 1N HCl to 1 g of sample, followed by vortexing for 2 minutes. For soy milk, 900 µL of 1N HCl was added to 100 µL of soy milk followed by vortexing for 2 minutes. After vortexing, samples were incubated for 5 minutes at room temperature, thereafter 1 mL of the sample supernatant was retained and centrifuged for 5 minutes at 6,000 rpm. After centrifugation, samples were diluted by adding 40 µL of sample to 4 mL of glyphosate sample diluent (supplied in the kit) followed by vortexing for 20 seconds. Sample derivatization followed by the addition of 1 mL of glyphosate assay buffer (supplied with the kit) and 100 µL of diluted glyphosate derivatization reagent (supplied with the kit) to 250 µL of sample, standards and control. Thereafter, the samples were vortexed for 20 seconds and the mixture incubated for 10 minutes at room temperature. The samples were tested in triplicate and each assay included five standards (0, 0.075, 0.2, 0.5, 1 and 4 parts per billion) and a control (0.75 parts per billion) in duplicate. Each standard/control/sample (50 µL) was added to the microtiter plate, followed by the addition of 50 µL glyphosate antibody solution (supplied in the kit). The plate was

incubated on a rotary shaker at 100 rpm at room temperature for 30 minutes. After incubation, 50 μL of glyphosate conjugate solution (supplied with the kit) was added to each well followed by incubation on a rotary shaker at 100 rpm at room temperature for 60 minutes. After incubation, the content of the plate was discarded and each well was washed three times by the addition of 250 μL of 1x wash buffer (supplied in the kit). Colour solution (150 μL) (supplied in the kit) was then added to each well and the plate incubated at room temperature for 20 minutes. After incubation, 100 μL of stop solution (supplied in the kit) was added to each well. The absorbance was read within 15 minutes of adding the stop solution, using the BioTek Synergy HT Plate Reader at 450 nm. Compensation for background noise was done by subtracting the mean optical density of the blank (zero standard) containing only reagent, from each sample reading. The standards were used to generate a Four Parameter Logistic curve ($R^2 > 0.98$) (Appendix C), which was used to determine the glyphosate concentration in each sample. Samples with a mean result above the highest standard were diluted as necessary and the assay repeated. The assay had a limit of detection (LOD) of 0.075 parts per billion (ppb) and samples containing glyphosate at levels below the LOD were considered negative according to the manufacturer's instructions for analysis.

2.6 Methodology for DNA screening

2.6.1 Sample preparation and DNA extraction

Samples were homogenized (where necessary), using a food blender to a maximum particle size of 2.5 mm². DNA extraction was performed in duplicate from homogenized samples using the cetyltrimethylammonium bromide (CTAB) method with some modification (Lipp *et al.*, 1999). Each extraction included an extraction control containing only reagents to ensure that no reagents were contaminated. DNA was extracted from duplicate 2 g samples by the addition of 10 mL CTAB (pH 8.0) [0.11M CTAB, 0.03M EDTA, 2.8M NaCl, 0.2M Tris] and 30 μL proteinase K [20 mg/mL]. After incubation at 60°C for at least 2 hours, 1.5 mL sample/CTAB solution was added to 50 μL RNase [20 mg/mL] and further incubated at 60°C for 15 minutes. Following this, 900 μL of sample supernatant was added to 600 μL of chloroform and the mixture was vortexed and then centrifuged at 14,000 rpm for 10 minutes. The

aqueous phase was retained to which 500 μL isopropanol and 2 μL glycogen was added, followed by incubation for 30 minutes at room temperature to precipitate the DNA. The DNA pellet was retained and washed with 500 μL of 75% ethanol followed by centrifugation at 14,000 rpm for 5 minutes. The DNA was then dissolved in 50 μL of 0.1x TE buffer (pH 8.0) [1M Tris and 0.5M EDTA]. The DNA was further purified using Eurofins GeneScan micro-spin columns according to the manufacturer's instructions. The extracted DNA was stored at 4°C until used.

2.6.2 Gel electrophoresis and fluorometry

Samples negative for an HT event were subjected to agarose gel electrophoresis in order to exclude the possibility of a false negative result due to a failed DNA extraction. Samples that screened positive for GM HT events were not subjected to electrophoresis, but quantified and the quality of extracted DNA was evaluated by the copy number of the *High Mobility Group (HMG)* (for maize) or *Lectin* (for soybean) gene. Sample DNA (5 μL) was added to 10 μL of blue dye loading buffer [60% Glycerol, 0.5M EDTA (8.0 pH) and bromophenol blue] in duplicate and gel electrophoresis performed using a 1% agarose gel in sodium borate (SB) buffer (pH 8.0) [10mM NaOH, 30mM boric acid]. Lambda DNA (7 μL) [50 ng/ μL] was used to evaluate the size of the extracted DNA. The agarose gel was run at 250V for 15 to 20 minutes, followed by staining in ethidium bromide solution [10 mg/mL ethidium bromide] on a rotary shaker at 50 rpm for 15 minutes. The DNA was visualized and documented under UV light using the GelLogic200 (Kodak) system.

The concentration of extracted DNA in products that tested negative for GM HT events but that contained glyphosate was determined fluorometrically using the Qubit dsDNA HS Assay Kit (Invitrogen) according to the manufacturer's instructions. Two calibration standards, at 0 ng/ μL and 10 ng/ μL respectively, were prepared by the addition of 10 μL of each standard to 189 μL of Qubit dsDNA HS Buffer and 1 μL of Qubit dsDNA HS Reagent provided in the kit. The concentration of DNA was determined by the addition of 1 μL of extracted DNA to 198 μL of Qubit dsDNA HS Buffer and 1 μL Qubit dsDNA HS Reagent. The mixture was vortexed and centrifuged, followed by

incubation at room temperature for 2 minutes before measuring the concentration of DNA using the Qubit fluorometer (Invitrogen).

2.6.3 Real-time PCR screening for the presence of GM HT events in food products

The extracted DNA was used to screen samples for the presence of glyphosate tolerant events approved in South Africa for maize and soybean, respectively. Event specific qualitative Real-time PCR detection was used to screen for events NK603 and GA21 in maize and event GTS40-3-2 in soybean. The Real-time PCR reaction mixture consisted of 5 μ L DNA and 20 μ L of Real-time PCR master mix (7.5 μ L oligo mix and 12.5 μ L basic mix) (Eurofins GeneScan, Germany). A synthetic PCR target was included in the Real-time PCR master mix reaction as internal inhibition control for each sample. All reactions were performed in duplicate including positive, negative and extraction controls. An inhibition control was included Samples as well as all the positive, negative and extraction controls were subjected to the following thermal cycling conditions: an initial denaturation and enzyme activation step of 95°C for 10 minutes, followed by 45 cycles of 95°C for 15 seconds and 60°C for 90 seconds. The real-time PCR reaction was performed on an Mx3005P Cyclor (Agilent Technologies) with a LOD of 10 copies. Fluorescence data was collected during the annealing/elongation step at 60°C. Products were scored for the presence or absence of the events, only if duplicate results were uniform. To minimize the risk of cross-contamination, individual steps including sample preparation, DNA extraction, PCR setup and PCR were performed in separate laboratories.

2.6.4 Real-time PCR quantification of GM HT events detected in food products

The GM HT event(s) found to be present in DNA samples was quantified by using the GMO Quant NK603 Corn and GMO Quant GA21 Corn kits for maize products and the GMO Quant RoundupReady Soy kit for soybean products, respectively (Eurofins GeneScan). The kits included real-time PCR master mixes with respective primers and probes, four copy number standards each for target and reference genes (Table

2.2 and 2.3), respectively and a 1% GM certified reference control (ERM-BF415d for NK603, ERM-BF414d for GA21 and ERM-BF410dn for GTS40-3-2) (JRC-IRMM, 2017). The copy number standards were used to generate a standard curve consisting of four data points in duplicate with a minimum correlation of 0.98 and a limit of quantification (LOQ) of 0.05%. The total NK603 and GA21 content in the maize products was quantified in relation to the *HMG* reference gene. The total GTS40-3-2 content in the soybean products was quantified in relation to the *Lectin* reference gene. The Real-time PCR thermal cycling conditions was as follows: an initial denaturation and enzyme activation step of 95°C for 10 minutes, followed by 45 cycles of 95°C for 15 seconds and 60°C for 90 seconds. The Real-time PCR reaction mixture consisted of 5 µL DNA and 20 µL of Real-time PCR master mix. Each reaction was performed on the ABI 7500 FAST Real-time PCR system (Applied Biosystems). Two dilutions of each sample in duplicate (1:2 and 1:8 for unprocessed samples; 1:4 and 1:8 for processed samples) were used to test for sample inhibition. In addition to this, a no template control was included with each assay to test for contaminated reagents. Products were considered to be negative for a GM HT event if the maize and soybean references gene was detected at sufficient copy number.

Table 2.2: Copy number standards used in the Real-time PCR quantification of maize events NK603 and GA21.

Standards	<i>HMG</i> (copies/5 µL)	NK603 and GA21 (copies/5 µL)
1	81,920 copies	10,240 copies
2	10,240 copies	1,280 copies
3	1,280 copies	160 copies
4	160 copies	40 copies

Table 2.3: Copy number standards used in the Real-time PCR quantification of soybean event GTS40-3-2.

Standards	<i>Lectin</i> (copies/5 µL)	GTS40-3-2 (copies/5 µL)
1	86,400 copies	8,600 copies
2	14,400 copies	1,440 copies
3	2,400 copies	240 copies
4	400 copies	40 copies

2.7 Data analysis

The mean amount of glyphosate and percentage of GM HT event present in each sample as well as the standard deviation was calculated using Excel 2010 (Windows 8). Linear regression was performed to determine whether a correlation existed between the percentage of GM HT DNA and level of glyphosate in each sample. To achieve consistency glyphosate concentrations (either in mg/kg or mg/L) were converted to mg/kg (by using the density of glyphosate 1.75 kg/L) (Dill *et al.*, 2010) to allow for comparison between different studies as well as to the MRL for glyphosate.

2.8 Compliance to mandatory GM labelling in South Africa

Data obtained from the quantification of GM HT events in the maize and soybean food products included in this study was used to check for compliance with regards to GM labelling. Products were evaluated in terms of current GM labelling requirements under the Consumer Protection Act (2008): “genetically modified” (above 5% GM), no GM labelling required (below 5% GM) or “non-GMO” (below 1% GM). This was done by comparing the percentage GM HT event to the GM label in the ingredients list of each product used in this study.

CHAPTER 3: RESULTS AND DISCUSSION FOR GLYPHOSATE CONTENT IN FOOD PRODUCTS

3.1 Detection of glyphosate in maize and soybean food products

Eighty-one off-the-shelf maize and soybean food products were tested for glyphosate. Of these products, 57 indicated maize as a primary constituent, 11 specified soybean as a primary constituent, while 13 contained both maize and soybean as a primary constituent (corn-soy blends and texturized soy protein products). Of all the products tested, 54 (66.70%) contained glyphosate in a range of 27 to 2,257 parts per billion (ppb) (0.027 to 2.26 mg/kg). Of the 57 maize products, 30 (52.63%) contained glyphosate in a range of 27 to 95 ppb (0.027 to 0.095 mg/kg) (Table 3.1). All 11 soybean products contained glyphosate in a range of 27 to 142 ppb (0.027 to 0.142 mg/kg) (Table 3.2). Of the six corn-soy blends, all tested positive for glyphosate in a range of 43 to 65 ppb (0.043 to 0.065 mg/kg) (Table 3.3). All seven texturized soy protein products tested positive for glyphosate in a range of 41 to 2,257 ppb (0.041 to 2.26 mg/kg) (Table 3.3). These findings confirm that glyphosate is present in South African food products containing maize and soybean.

The levels of glyphosate detected in the maize products in this study are low when compared to the MRL for glyphosate in maize in South Africa (2 mg/kg). The highest level of glyphosate detected in the maize products (95 ppb or 0.095 mg/kg) was 4.75% of the MRL (Table 1.1). Similarly, none of the soybean products contained levels of glyphosate above the MRL established for soybeans in South Africa (20 mg/kg) and the highest level detected (142 ppb or 0.14 mg/kg) was 0.71% of the MRL (Table 1.1). The levels of glyphosate detected in the corn-soy blends and texturized soy protein products (that contained maize and soybean) were compared to both the MRLs for maize and soybean respectively, since there is no MRL for glyphosate in composite and/or processed products. The highest level of glyphosate detected in the corn-soy blends (65 ppb or 0.065 mg/kg) was below the MRL for maize and soybean (3.25% of the MRL for glyphosate in maize and 0.33% of the MRL for glyphosate in soybean). The highest level of glyphosate detected in texturized soybean protein (containing both maize and soybean) (2,257 ppb or 2.257 mg/kg) exceeded the MRL in maize but was

below the MRL in soybean (112.85% of the MRL for glyphosate in maize and 11.30% of the MRL for glyphosate in soybean). No other texturized soy protein products contained levels of glyphosate exceeding the MRLs for maize or soybean. With the exception of one texturized soy protein product, the level of glyphosate in all products included in this study was below the MRLs established for maize and soybean in South Africa. The results from this study suggest that the correct application rate of glyphosate is practiced in South African agriculture.

As previously stated in this dissertation, the MRL for a pesticide is based on agricultural practice. The MRL for glyphosate in soybean is 10 fold higher than maize simply due to the fact that HT soybean is treated more frequently with the herbicide during the growing season than HT maize. Over the last two decades the MRL for glyphosate in soybean has been increased several times. Prior to 1999 the MRL for glyphosate in soybean in the USA and Europe was 0.1 mg/kg. In 1999, it was increased 200 fold in the USA and Europe to 20 mg/kg. Bøhn *et al.* (2014) suggested that the MRL for glyphosate in soybean was increased due to the higher levels of herbicide in this commodity. These changes confirm the inconsistency when establishing the MRL for a pesticide. Although the level of glyphosate detected in the maize and soybean food products in this study was below the MRL, it should be noted that a MRL cannot be considered as an indication of safety.

Table 3.1: Summary of the glyphosate content in maize food products.

Sample category	Number of Samples	Number of samples testing positive for glyphosate	Range of glyphosate in samples (ppb)	Range of glyphosate in samples (mg/kg)
Maize meal	20	20	27 to 93	0.027 to 0.093
Instant maize meal	2	2	34 to 35	0.034 to 0.035
Beer powder	2	2	40 to 95	0.040 to 0.095
Maize grits	5	0	ND	ND
Maize rice	3	3	28 to 65	0.028 to 0.065
Polenta	5	0	ND	ND
Corn flakes	7	0	ND	ND
Corn chips	10	0	ND	ND
Maize pasta	3	3	47 to 62	0.047 to 0.062

ND: Not detected

Table 3.2: Summary of the glyphosate content in soybean food products.

Sample category	Number of samples	Number of samples testing positive for glyphosate	Range of glyphosate in samples (ppb)	Range of glyphosate in samples (mg/kg)
Soy milk	8	8	32 to 142	0.032 to 0.142
Soybeans	1	1	49	0.049
Infants milk	1	1	58	0.058
Soy flour	1	1	27	0.027

Table 3.3: Summary of the glyphosate content in texturized soy protein and corn-soy blend food products.

Sample category	Number of samples	Number of samples testing positive for glyphosate	Range of glyphosate in samples (ppb)	Range of glyphosate in samples (mg/kg)
Texturized soy protein	7	7	195 to 2,257	0.195 to 2.257
Corn-soy blend	6	6	43 to 65	0.043 to 0.065

3.2 Detection and quantification of GM HT events in food products

The majority of maize and soybean food products (70.37%) contained one or more GM HT event. Of the 57 maize products, 44 (77.19%) tested positive for at least one GM HT maize event (NK603 and/or GA21) (Table 3.4). The most common GM HT maize event was NK603, detected in 44 out of 57 maize products (77.19%) in a range of 0.25% to 100.00%. GM HT maize event GA21 was detected in only one maize product at 0.72% (Table 3.4). The higher incidence of event NK603 compared to event GA21 is not surprising, since event NK603 was introduced for commercial production in South Africa in 2002 whereas event GA21 was only introduced for planting in 2010 (ISAAA, 2015). For soybean, GTS40-3-2 is the only GM HT event approved in South Africa. Of the 11 soybean products included in this study, three (27.27%) tested positive for the GM HT soybean event GTS40-3-2 in a range of 0.07% to 9.57% (Table 3.5). The corn-soy blends and texturized soy protein products were tested, for both maize and soybean events, according to their ingredient list. Of the six corn-soy blends, five (83.33%) tested positive for NK603 and/or GTS40-3-2. Event NK603 was detected in three out of six corn-soy blends in a range of 0.05% to 16.70% (Table 3.6). Event GTS40-3-2 was detected in three out of six corn-soy blends in a range of 0.14% to 48.65% (Table 3.7). Of the seven texturized soy protein products, five (71.43%) tested positive for NK603 and/or GTS40-3-2. Event NK603 was detected in three of the seven texturized soy protein products in a range of 27.75% to 54.38%, while GTS40-3-2 was present in five out of seven texturized soy protein products in a range of 16.75% to 92.09% (Table 3.6 and Table 3.7). Three products contained GM HT events at below the LOQ (0.05%) (Appendix B). No GM HT event was detected in 21 out of 81 food products (25.9%) (Appendix B).

Table 3.4: Detection and quantification of NK603 and GA21 in maize food products.

Sample category	Number of samples	Number of samples containing HT events	Number of samples containing event NK603	Number of samples containing event GA21	Percentage range of NK603 in samples	Percentage range of GA21 in samples
Maize meal	20	19	19	1	1.28% to 100.00%	0.72
Instant maize meal	2	1	1	0	80.61%	ND
Beer powder	2	2	2	0	26.24% to 40.47%	ND
Maize grits	5	5	5	4	16.39% to 55.18%	Below LOQ
Maize rice	3	3	3	1	9.72% to 67.47%	Below LOQ
Polenta	5	4	4	0	0.25% to 53.65%	ND
Corn flakes	7	0	0	0	ND	ND
Corn chips	10	9	9	0	10.76% to 41.65%	ND
Maize pasta	3	1	1	0	21.15%	ND

Below LOQ: refers to a GM HT event being detected, but below 0.05%

ND: Not detected

Table 3.5: Detection and quantification of GTS40-3-2 in soybean food products.

Sample category	Number of samples	Number of samples containing HT event GTS40-3-2	Percentage range of GTS40-3-2 detected
Soybeans	1	0	ND
Soy milk	8	2	5.21% to 9.57%
Infants milk	1	1	0.07%
Soy flour	1	0	ND

ND: Not detected

Table 3.6: Detection and quantification of NK603 in texturized soy protein and corn-soy blend food products.

Sample category	Number of samples	Number of samples containing HT event NK603¹	NK603 detected
Corn-soy blend	6	3	0.04% to 16.70%
Texturized soy protein	7	3	27.75% to 54.38%

¹ Event NK603 was the only HT maize event detectable in texturized soy protein products and corn-soy blends tested in this study, while event GA21 was not detected in these products

Table 3.7: Detection and quantification of GTS40-3-2 in texturized soy protein and corn-soy blend food products.

Sample category	Number of samples	Number of samples containing HT event GTS40-3-2	GTS40-3-2 detected
Corn-soy blend	6	3	0.14% to 48.65%
Texturized soy protein	7	5	16.75% to 92.09%

3.3 Correlation between percentage GM HT event and level of glyphosate in food products

Since the products were tested for both glyphosate and GM HT events, a linear regression was used to evaluate whether a correlation existed between the percentage GM HT event and level of glyphosate. There was no correlation between the percentage GM HT event(s) and the level of glyphosate ($R^2 = 0.17$). Moreover, some of the products that contained glyphosate did not contain detectable GM HT events. It is possible that glyphosate was used as a desiccant on crops that did not contain an HT event (Duke *et al.*, 2003; IARC, 2015). It is also possible that the products that tested negative for GM HT events were false HT negative as a result of processing thereby degrading the DNA. After further analysis, by excluding products that tested negative for GM HT events, still no correlation was observed ($R^2 = 0.16$). Thus, it can be concluded that there is no correlation between the GM HT content and the level of glyphosate in a product.

Of the 81 products analysed for glyphosate content, 12 contained glyphosate in a range of 27 ppb to 61 ppb (0.027 mg/kg to 0.061 mg/kg) even though they did not contain a detectable GM HT event (Table 3.8). As previously stated, products that tested negative for GM HT events could have been as a result of DNA degradation during food processing (Bauer *et al.*, 2003). However, the products that tested negative for GM HT events, including an instant maize meal, maize meal, soybeans and soy flour, for which there was detectable DNA after gel electrophoresis, suggest that these products were not false negative due to processing (Figure 3.1). Furthermore, PCR inhibition can also be excluded for samples testing negative for GM HT events, since all products were tested at two dilutions. The remaining samples consisted of two maize pastas, one corn-soy blend, four soy milks and a texturized soy protein product from which little or no visible DNA was evident after gel electrophoresis (Figure 3.2). Considering the sensitivity of agarose gel electrophoresis it is possible that low amounts of degraded DNA were present in these samples and fluorometry was used to determine the concentration of DNA (Table 3.9). The highest concentration of DNA in these samples was 0.449 ng/ μ L which is low and may explain the negative PCR results. A further three products contained event

GTS40-3-2 at below the LOQ (0.05%) but had a level of glyphosate in a range of 32 ppb to 40 ppb (0.032 mg/kg to 0.040 mg/kg) (Table 3.10). The Real-time PCR quantification results indicated that the *Lectin* gene was present in sufficient copy numbers in these samples. This confirmed that these products contained low amounts of the event GTS40-3-2 (Table 3.10).

Some products tested positive for a GM HT event(s) but did not contain any glyphosate. It was found that 21 products contained GM HT events in a range of 0.25% to 67.47%, without containing glyphosate (Table 3.11). Since glyphosate is not removed from grain or food by washing, cooking or processing (EFSA, 2009), it is likely that the GM HT crops used to produce these products was not treated with the herbicide. The majority of maize and soybean produced in South Africa is cultivated on dryland. Thus, the emergence of weeds (and hence the need to apply glyphosate) would depend on several factors including rainfall and/or agricultural practice. These results suggest that the application of glyphosate is likely to differ from season to season.

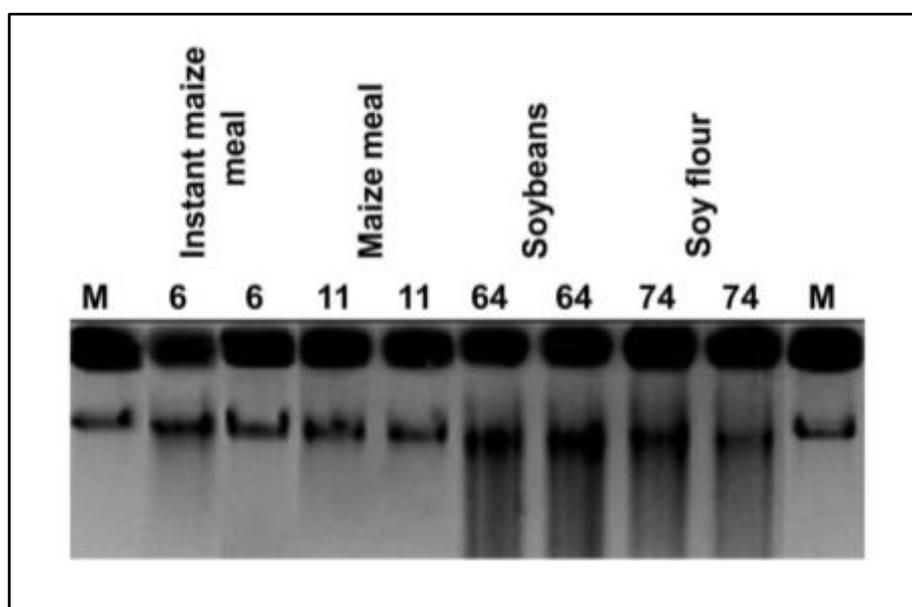


Figure 3.1: Negative inverted 1% agarose gel image of GM HT negative samples with visible DNA. The extracted DNA for samples 6 (instant maize meal), 11 (maize meal), 64 (soybeans) and 74 (soy flour) in duplicate were resolved on a 1% agarose gel. All samples indicated visible DNA. Lambda DNA [50 ng/ μ L] (lanes marked M) was used to evaluate the quality of the extracted sample DNA.

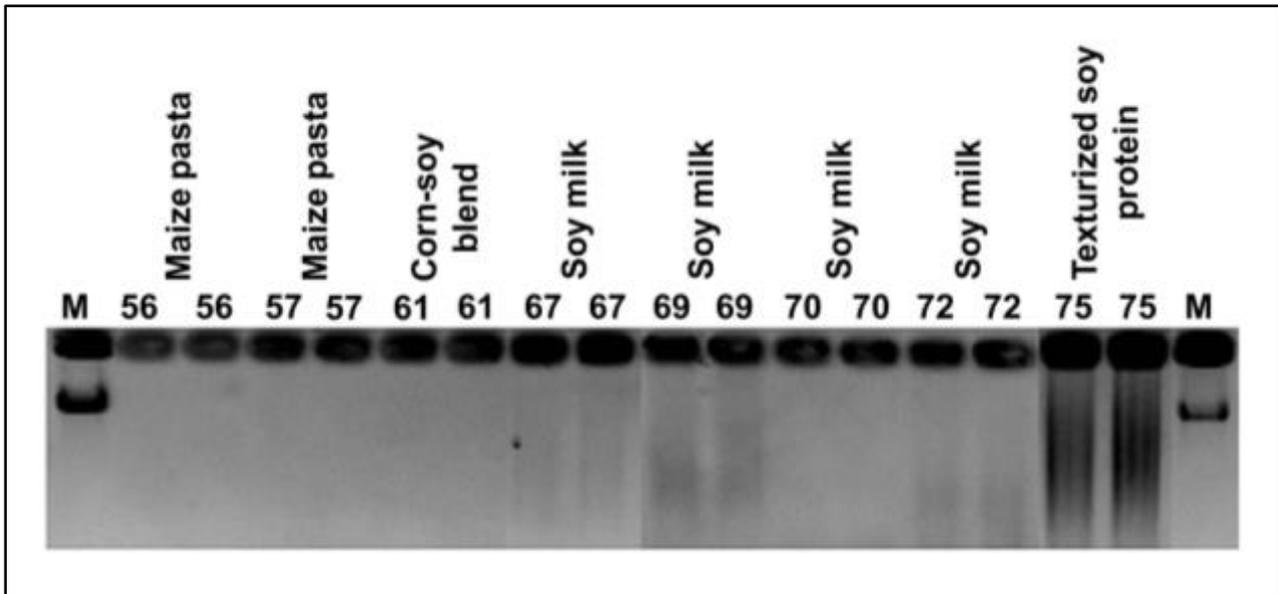


Figure 3.2: Negative inverted 1% agarose gel image of GM HT negative samples with little or no visible DNA. The extracted DNA for samples 56 (maize pasta), 57 (maize pasta), 61 (corn-soy blend), 67 (soy milk), 69 (soy milk), 70 (soy milk), 72 (soy milk) and 75 (texturized soy protein) in duplicate were resolved on a 1% agarose gel. No DNA was visible for samples 56 (maize pasta), 57 (maize pasta), 61 (corn-soy blend) and 70 (soy milk). Samples 67 (soy milk), 69 (soy milk), 72 (soy milk) and 75 (texturized soy protein) indicated smears, suggesting that degraded DNA may be present. Lambda DNA [50 ng/μL] (lanes marked M) was used to evaluate the quality of extracted sample DNA.

Table 3.8: Food products that contained glyphosate but no GM HT event.

Sample identification number	Description	Glyphosate residue (ppb)	Glyphosate residue (mg/kg)
6	Instant maize meal	49	0.049
11	Maize meal	34	0.034
56	Maize pasta	47	0.047
57	Maize pasta	50	0.050
61	Corn-soy blend	61	0.061
64	Soybeans	49	0.049
67	Soy milk	36	0.036
69	Soy milk	52	0.052
70	Soy milk	49	0.049
72	Soy milk	50	0.050
74	Soy flour	27	0.027
75	Texturized soy protein	41	0.041

Table 3.9: Fluorometric determination of DNA concentration in GM HT negative samples.

Sample identification number	DNA concentration in duplicate (ng/μL)	Mean DNA concentration (ng/μL)
56	0.145	0.172
	0.198	
57	0.129	0.142
	0.155	
61	0.254	0.270
	0.285	
67	0.009	0.008
	0.007	
69	0.005	0.007
	0.009	
70	0.014	0.010
	0.005	
72	0.003	0.005
	0.006	
75	0.453	0.449
	0.445	

Table 3.10: Food products containing GM HT event GTS40-3-2 below the limit of quantification but containing glyphosate.

Sample identification number	Description	GM status	Copy number of reference genes (<i>HMG</i> and <i>Lectin</i>)	Glyphosate residue (ppb)	Glyphosate residue (mg/kg)
66	Soy milk	Below LOQ	15,464 copies of <i>Lectin</i>	40	0.040
68	Soy milk	Below LOQ	20,654 copies of <i>Lectin</i>	32	0.032
79	Texturized soy protein	Below LOQ	18,402 copies of <i>Lectin</i>	37	0.037

Table 3.11: Food products containing GM HT event NK603 but with no detectable glyphosate.

Sample identification number	Description	Percentage of GM HT event detected
25	Maize grits	54.63%
26	Maize grits	42.51%
27	Maize grits	55.18%
28	Maize grits	16.39%
29	Maize grits	21.50%
30	Maize grits	67.47%
31	Maize grits	49.41%
32	Maize grits	9.72%
33	Polenta	0.25%
34	Polenta	53.65%
35	Polenta	11.55%
36	Polenta	31.56%
45	Corn chips	11.54%
46	Corn chips	41.65%
47	Corn chips	33.15%
48	Corn chips	35.33%
49	Corn chips	10.76%
50	Corn chips	20.36%
51	Corn chips	17.54%
52	Corn chips	16.35%
53	Corn chips	12.22%

3.4 Theoretical exposure to glyphosate through food products in South Africa

In this study, glyphosate was detected in 66.70% of the food products analysed. The level of glyphosate in the food products was compared to the acute toxicity level as well as to the no observed adverse effect level (NOAEL) established for glyphosate in experimental animals. Acute toxicity refers to the adverse effects of a substance occurring after a single dose or from multiple doses within 24 hours (Rand, 1995), while the NOAEL refers to the highest dose of a substance at which no adverse or toxic effects are observed (Darota and Engelhardt, 2005). The acute toxicity level for glyphosate in experimental animals is suggested to be approximately 5,000 mg/kg, while the NOAEL is 175 mg/kg (USA EPA, 2002). Thus, the acute toxicity level of glyphosate for a 60 kg individual (considered to be the mean weight of individuals from Africa (Walpole *et al.*, 2012)) will be 300,000 mg, while the NOAEL will be 10,500 mg. Based on these data, the level of glyphosate in the food products tested is considered safe.

The safety of food additives or pesticide residue is based on the NOAEL based on animal studies that has been shown to cause no adverse effect (USA FDA, 1993). The NOAEL is then used to determine the acceptable daily intake (ADI) based on an appropriate safety factor (USA FDA, 1993). The ADI refers to the amount of a substance that a consumer can ingest daily over an entire lifetime without any recognizable health risk (Benford, 2000). It is important to note that the NOAEL and ADI is based on the safety of pure glyphosate, which compared to glyphosate in formulation, is not very permeable into the cell. The ADI for glyphosate differs between countries and ranges from 0.3 mg/kg in Europe to 1.75 mg/kg in the USA (FAO, 2013). The ADI for glyphosate in South Africa is 1 mg/kg of bodyweight per day (ACB, 2012). The theoretical intake of glyphosate resulting from the consumption of the maize and soybean products in this study was calculated and compared to the ADI (Table 3.12). The theoretical intake of glyphosate, as a result of consuming the maize products tested in this study, ranged from 0.001 mg to 0.047 mg, which is below the ADI (Table 3.12). For the soybean products, the theoretical intake of glyphosate ranged from 0.003 mg to 0.021 mg that is also below the ADI (Table 3.12). One of the texturized soy protein products contained a level of glyphosate of 2.257 mg/kg, which would amount to a theoretical intake of 0.339 mg (Table 3.12). Thus, the intake of glyphosate

as a result of the daily consumption of the maize and soybean products tested in this study does not exceed the ADI in South Africa.

It is important to consider that maize is the primary staple and soybean an important source of protein in South Africa. A study by Payne (2011) suggested that approximately 500 g of maize meal is consumed daily by adults (age 18 to 65) in poor households. Based on this, it is estimated that 182 kg of maize meal is consumed per person annually and approximately 8,601 kg over the average adult lifespan (age 18 to 65). Considering that the level of glyphosate detected in maize meal, in this study, ranged from 0.027 to 0.093 mg/kg (Table 3.1), the total exposure to glyphosate would be approximately 5 mg to 17 mg annually and approximately 240 mg to 806 mg over the average adult lifespan (age 18 to 65). A recent report by the National Agricultural Marketing Counsel of South Africa (NAMC) indicated that texturized soy protein was the major soybean food product consumed in South Africa, accounting for approximately 52% of the soybean food market (NAMC, 2011). In the absence of an estimation of the daily intake of soybean per adult individual in South Africa, the serving suggestion was used to calculate the maximum potential consumption. Based on this, it is estimated that 54.8 kg of texturized soy protein is in theory consumed per person annually and approximately 2,575.6 kg (54.8 x 47 years) over the adult lifespan (age 18 to 65). Considering that the level of glyphosate detected in texturized soy protein products, in this study, ranged from 0.041 to 2.257 mg/kg (Table 3.3), the total exposure to glyphosate would be approximately 2.2 mg to 123.7 mg annually and approximately 105.6 mg to 5,813.1 mg over the average adult lifespan (age 18 to 65). This study suggests that the consumption of maize and soybean food products does expose South Africans to glyphosate but at low levels.

Table 3.12: Theoretical daily intake of glyphosate through food products.

Product category	Maximum daily product consumption (g)	Range of glyphosate in product (mg/kg)	Daily glyphosate intake (mg)	% of SA ADI for a 60kg adult (60 mg)
Maize meal	500 ¹	0.027 to 0.093	0.014 to 0.047	0.023 to 0.078
Beer powder	100 ²	0.040 to 0.095	0.004 to 0.01	0.007 to 0.017
Maize rice	50 ²	0.028 to 0.065	0.001 to 0.003	0.002 to 0.005
Maize pasta	40 ²	0.047 to 0.062	0.002 to 0.002	0.003 to 0.003
Soybean milk	150 ²	0.032 to 0.142	0.005 to 0.021	0.008 to 0.035
Soybeans	100 ²	0.049	0.005	0.008
Infants Milk	144 ²	0.058	0.008	0.013
Soy Flour	100 ²	0.027	0.003	0.005
Corn-soy blend	30 ²	0.043 to 0.065	0.001 to 0.002	0.002 to 0.003
Texturized soy protein	150 ²	0.041 to 2.257	0.006 to 0.339	0.01 to 0.565

1 For maize meal, 500 g per person per day was used as estimated by Payne (2011)

2 The maximum daily intake was estimated from the serving suggestion on each packet

In recent years, several *in vitro* studies on human cell lines have reported that glyphosate in formulation exhibits toxicity at low concentrations. These findings include human cell death at 5 mg/kg as well as DNA damage and endocrine disruption at concentrations ranging from 0.006 mg/kg to 0.5 mg/kg (Table 1.3). Taking this into account, the levels of glyphosate detected in the maize and soybean products in this study cannot be summarily considered to have no effect. Of the 54 products containing glyphosate, three contained above 0.5 mg/kg (500 ppb) (Appendix A) that has been determined to cause endocrine disruption in cultured humans cells (Gasnier *et al.*, 2009). A further 12 products contained glyphosate above 0.069 mg/kg (69 ppb) (Appendix A) that has been determined to cause DNA damage in human lymphocytes (Alvarez-Moya *et al.*, 2014). Furthermore, all 54 products that were positive for glyphosate contained above 0.006 mg/kg (6 ppb) (Appendix A), that was determined to cause chromosomal breakage in hamster ovary cells (Roustan *et al.*, 2014). In addition to this, the IARC recently re-classified glyphosate as a “probable human carcinogen”. In conclusion, although considered safe based on the ADI as well as the acute toxicity level and NOAEL, the effect of exposure to glyphosate at low levels through maize meal, consumed daily in South Africa, warrants further research.

CHAPTER 4: RESULTS AND DISCUSSION IN TERMS OF COMPLIANCE WITH GM LABELLING IN SOUTH AFRICA

4.1 Analysis of GM HT content in GM labelled food products

The percentage of GM HT event was quantified in GM HT positive products and most of these were labelled in terms of GM content. Since the data was available, it was used to determine compliance to mandatory GM labelling in terms of the Consumer Protection Act (2008) that mandates GM labelling in South Africa. According to section D: 24 of the Act, “any person who produces, supplies, imports or packages any prescribed goods must display on, or in association with the packaging of those goods, a notice in the prescribed manner and form that discloses the presence of any genetically modified ingredients or components of those goods in accordance with applicable regulations” (SACPA, 2009). In South Africa, the application of mandatory GM labelling is as follows (Viljoen and Marx, 2013):

- Ingredients in food containing more than 5% GM must be labelled “genetically modified”.
- Ingredients in food containing less than 1% GM may voluntarily be labelled “non-GMO”.
- Companies may use a cost effective option of labelling food ingredients “may contain genetically modified ingredients” where GM testing is not scientifically practical or feasible.

Although the food products were only tested for GM HT events (NK603, GA21 and GTS40-3-20), a product was considered not to comply with the Consumer Protection Act if it was labelled as non-GM but contained more than 1% of a GM HT event or was not labelled to contain GM and contained above 5% of a GM HT event. Of the 81 food products, 57 had a label indicating GM status. Of the 57 labelled products, 28 were labelled “contains GMO”, 14 were labelled “may contain GMO”, five were labelled “non GMO” and ten were labelled “GMO free”. Of the 28 products labelled “contains GMO”, 25 contained a GM HT event above 5%, while 3 contained a GM HT event below 1% (Table 4.1). Of the 14 products labelled “may contain GMO”, 12 contained a GM HT event above 5%, while two products contained a GM HT event below 5% (Table 4.1). Based on the percentage GM in these products, it is evident that mandatory GM

labelling has not had a negative impact on the GM market as initially feared by the agricultural industry (Viljoen and Marx, 2013). In terms of labelling to indicate the absence of GM, five products were labelled “non-GMO” and all of these products contained a GM HT event below 1% (Table 4.2). In addition to this, ten products were labelled “GMO free”, of which four products contained a GM HT event above 5%, one contained a GM HT event above 1%, and one contained a GM HT event below 1% (Table 4.2). “GMO free” is not legally defined by the South African Consumer Protection Act (2008) and is not internationally accepted as it cannot be defined analytically (Viljoen *et al.*, 2006). The term “GMO free” implies 0% GM content and as stated above six products labelled “GMO free” contained a GM HT event which ranged from 0.25% to 53.65% (Table 4.2). Therefore, the use of “GMO free” in these products is misleading and inaccurate. From this, it can be concluded that discerning consumers with a preference for non-GM products will be more likely to get what they expect from a “non GMO” labelled product than from products labelled “GMO free”. Overall, the results from this study indicate that the majority of companies comply with the Consumer Protection Act in terms of GM labelling with only a few incidences of mislabelling. Since only GM HT events were tested in this study it is important to note that insect resistant events may be present (only for maize products) resulting in an underestimation of the GM content. Nonetheless, constant monitoring of compliance to the Consumer Protection Act in terms of GM labelling is important to protect the rights of consumers in South Africa.

Table 4.1: GM HT quantification and GM label.

Sample identification number	Sample description	GM related claim on product	Percentage GM content
1	Maize meal	May contain GMO	92.34% ^{NK603}
2	Maize meal	May contain GMO	100.00% ^{NK603}
3	Maize meal	May contain GMO	45.78% ^{NK603}
4	Maize meal	Contains GMO	61.60% ^{NK603}
5	Maize meal	Contains GMO	100.00% ^{NK603}
7	Maize meal	Contains GMO	59.90% ^{NK603}
8	Maize meal	Contains GMO	35.80% ^{NK603}
9	Maize meal	Contains GMO	76.27% ^{NK603}
10	Maize meal	Contains GMO	32.17% ^{NK603}
13	Maize meal	Contains GMO	32.55% ^{NK603}
14	Maize meal	Contains GMO	30.45% ^{NK603}
15	Maize meal	May contain GMO	6.44% ^{NK603}
16	Maize meal	May contain GMO	35.97% ^{NK603}
17	Maize meal	May contain GMO	4.77% ^{NK603}
18	Maize meal	Contains GMO	24.95% ^{NK603}

Table 4.1: (continued)

Sample identification number	Sample description	GM related claim on product	Percentage GM content
20	Maize meal	Contains GMO	80.61% ^{NK603}
19	Maize meal	Contains GMO	22.81% ^{NK603}
21	Maize meal	Contains GMO	27.07% ^{NK603} and 0.72% ^{GA21}
22	Maize meal	Contains GMO	26.62% ^{NK603}
24	Beer powder	May contain GMO	40.47% ^{NK603}
25	Maize grits	May contain GMO	54.63% ^{NK603}
26	Maize grits	Contains GMO	42.51% ^{NK603}
27	Maize grits	Contains GMO	55.18% ^{NK603}
28	Maize grits	Contains GMO	16.39% ^{NK603}
29	Maize grits	May contain GMO	21.50% ^{NK603}
30	Maize grits	May contain GMO	67.47% ^{NK603}
31	Maize grits	Contains GMO	49.41% ^{NK603}
32	Maize grits	May contain GMO	9.72% ^{NK603}
38	Corn flakes	Contains GMO	ND
42	Corn flakes	Contains GMO	ND

Table 4.1: (continued)

Sample identification number	Sample description	GM related claim on product	Percentage GM content
45	Corn chips	Contains GMO	11.54% ^{NK603}
46	Corn chips	Contains GMO	41.65% ^{NK603}
47	Corn chips	Contains GMO	33.15% ^{NK603}
48	Corn chips	Contains GMO	35.33% ^{NK603}
49	Corn chips	Contains GMO	10.76% ^{NK603}
50	Corn chips	Contains GMO	20.36% ^{NK603}
51	Corn chips	Contains GMO	17.54% ^{NK603}
52	Corn chips	May contain GMO	16.35% ^{NK603}
53	Corn chips	May contain GMO	12.22% ^{NK603}
55	Maize pasta	Contains GMO	21.15% ^{NK603}
58	Corn-soy blend	Contains GMO	Below LOQ
63	Corn-soy blend	May contain GMO	4.01% ^{GTS40-3-2}

Below LOQ: refers to a GM HT event being detected, but below 0.05%

ND: Not detected

Table 4.2: GM HT quantification of food products labelled “non-GMO” and “GMO free”.

Sample identification number	Sample description	GM related claim	Percentage GM content
11	Maize meal	GMO free	ND
12	Maize meal	GMO free	1.28% ^{NK603}
33	Polenta	GMO free	0.25% ^{NK603}
4	Polenta	GMO free	53.65% ^{NK603}
56	Maize pasta	GMO free	ND
59	Corn-soy blend	Non-GMO	0.12% ^{NK603}
61	Corn-soy blend	GMO free	ND
64	Soybeans	GMO free	ND
65	Soy milk	GMO free	9.57% ^{GTS40-3-2}
68	Soy milk	Non-GMO	Below LOQ
71	Soy milk	GMO free	5.21% ^{GTS40-3-2}
72	Soy milk	GMO free	ND
74	Soybean flour	Non-GMO	ND
75	Texturized soy protein	Non-GMO	ND
79	Texturized soy protein	Non-GMO	Below LOQ

Below LOQ: refers to a GM HT event being detected but below 0.05%

ND: Not detected

CHAPTER 5: LIMITATIONS OF THIS STUDY

There are inherent limitations associated with this study that should be considered when evaluating the data presented:

- The Abraxis Glyphosate Plate Assay is specific for glyphosate and does not detect AMPA which is a metabolic by-product of glyphosate. AMPA has only been detected in HT soybean and is not considered a metabolite in maize. Thus, the amount of glyphosate in soybean may be underestimated due to its metabolic conversion to AMPA.
- Since only GM HT events were quantified in this study, it is possible that the GM content of the products may be underestimated. However, this is only applicable to the maize products that may contain insect resistant events. The only event approved for GM soybean in South Africa is GTS40-3-2.
- The agricultural application rate of glyphosate may vary from season to season. Considering that most farming in South Africa is on dryland, season to season variation in rainfall will influence the extent of weed emergence and subsequently the application of glyphosate that would affect the amount of herbicide present in food products.
- It is not known to what extent glyphosate in food is absorbed through the gastrointestinal tract in humans. It is possible but currently unknown whether glyphosate in a food matrix may be more easily absorbed, in the gastrointestinal tract. However, since this is unknown, it must be considered that without surfactant, the glyphosate may not be so readily absorbed.
- Although this study focused on glyphosate in maize and soybean food products, it should be noted that South Africa also produces GM HT cotton. Cotton seed oil is used in various food products. Furthermore, although South Africa does not plant GM HT wheat, it is common practice to desiccate wheat with glyphosate before harvesting. The exposure to glyphosate through cotton seed oil and wheat in food was not determined in this study.

CHAPTER 6: CONCLUSION

South Africa is considered a major GM crop producing country ranking ninth in the world in 2015. In South Africa, GM HT maize and soybean account for approximately 75% of GM crops planted. These crops are frequently treated with glyphosate during the growing season to kill weeds. Several studies have indicated the presence of glyphosate in HT grain as well as processed food products from major GM crop producing countries including the USA and Argentina. However, to date no such data exists regarding the prevalence of glyphosate in HT grain produced in South Africa. Maize, in the form of maize meal, is considered the major staple food in South Africa, with approximately 500 g consumed daily per adult in poor households, while soybean is an important source of protein. Thus, the aim of this study was to test South African food products containing maize and soybean as a primary constituent, for glyphosate residue. The results from the study have shown that glyphosate was present in the majority of maize and soybean food products (n = 54) in a range of 27 to 2,257 ppb (0.027 mg/kg to 2.257 mg/kg) confirming that South African consumers are exposed to glyphosate through their food.

Although the research on the presence of glyphosate in food products is limited, some studies have detected levels of up to 100 ppb (0.1 mg/kg) in bread from the UK and levels of up to 564 ppb (0.564 mg/kg) in soy sauce from the USA. Of the food products tested in this study, 49 contained levels of glyphosate below 100 ppb (0.1 mg/kg), while five contained levels exceeding 100 ppb (one soy milk at 142 ppb and four texturized soy protein products ranging from 195 ppb to 2,257 ppb). Furthermore, 51 products contained levels of glyphosate below 564 ppb (0.564 mg/kg), while three contained levels exceeding 564 ppb (three texturized soy protein products ranging from 921 ppb to 2257 ppb). This confirmed that the levels of glyphosate in South African maize and soybean food products were in a similar range compared to levels detected in food products from the UK and USA.

There was no correlation between the percentage GM HT event and the level of glyphosate present in a product. The lack of correlation suggests that although farmers may plant GM HT maize or soybean, they will most likely only spray herbicide

if it is necessary. The latter explains why some products did contain glyphosate. Furthermore, glyphosate is also used as a desiccant on conventional crops, hence its presence in non-GM grain. Since environmental factors like rainfall and agricultural practice influence the emergence of weeds, season to season variation of glyphosate application is expected. As a result of this, the level of glyphosate in food will differ from season to season.

The level of glyphosate, in the food products analysed in this study, was compared to the MRL for maize and soybean as well as the ADI established for South Africa. The data from this study has determined that the level of glyphosate in the food products was low and did not exceed the MRL or ADI. It should be noted that MRL are not a safety indication but purely serve as a guideline for food trade and “good” agricultural practice. ADI is based on the NOAEL (175 mg/kg) of pure glyphosate which, without surfactants is not very permeable across the cell membrane. In agriculture, glyphosate is applied as part of a formulation which contains surfactants to facilitate the absorption into plant cells. Arguably, glyphosate in formulation at a similar concentration would be more toxic than pure glyphosate. While it is not known what the effect is on absorption, once glyphosate is already in a food matrix, it is arguable that once absorbed into a biological system like a plant cell, transfer into animal and human tissue after consumption may occur more easily.

In recent years, research has reported that glyphosate in formulation at concentrations as low as 0.006 mg/kg (6 ppb) and 0.069 mg/kg (69 ppb) can cause DNA and chromosomal damage in animal and human cells lines (Alvarez-Moya *et al.*, 2014; Roustan *et al.*, 2014). Based on these findings, the glyphosate in South African maize and soybean food products are at a level, that may affect the health of consumers. All the glyphosate positive samples in this study contained levels above 0.006 mg/kg (6 ppb) that has been reported to cause chromosomal breakage in animal cells. Of these, 12 samples (five maize meals, one beer powder, two soy milks and five texturized soy protein products) contained levels of glyphosate above 0.069 mg/kg that has been determined to cause DNA damage in human cells. When considering these findings, it can be argued that the daily consumption of a major staple like maize meal chronically exposes consumers to glyphosate, that is considered a “probable human carcinogen” by the IARC, at levels known to be genotoxic to human and animal cells.

The percentage GM HT event in each sample was also used to evaluate products in terms of GM labelling requirement in South Africa. The South African Consumer Protection Act mandates the labelling of products containing more than 5% GM (labelled as “contains GMO” or “may contain GMO”), while it makes provision for non-GM labelling in products containing below 1% GM (labelled as “non-GMO”). The majority of products tested in this study (n=54) were labelled in terms of their GM content and complied with the GM labelling law as required by the South African Consumer Protection Act (2008). Most of the products labelled “may contain GMO” or “contains GMO”, with the exception of two cornflakes samples, contained a GM HT event. All the products labelled “non-GMO” contained a GM HT event below 1%. However, of the ten products labelled “GMO free”, four contained a GM HT event above 5%, one contained a GM HT event above 1% and one contained a GM HT event below 1%. “GMO free” is not legally defined in South Africa and the use of the term indicates a lack of analytical understanding as it refers to a 0% GM content. Considering that six of the products labelled “GMO free” contained a GM HT event, the use of the term “GMO free” should be reconsidered as it is interpreted inaccurately and may be misleading to consumers. The findings of this study suggest that the majority of companies comply with the Consumer Protection Act in terms of GM labelling, nonetheless frequent monitoring of food products in terms of GM labelling remain important to protect the rights of South African consumers.

This is the first study that has investigated the content of glyphosate in South African food products. The current study has confirmed that glyphosate is present in commercially available maize and soybean food products in South Africa. This does not imply that these food products are unsafe but that South African consumers are exposed daily to glyphosate through their diet. The question of whether the glyphosate in food is safe in the long term, needs to be addressed through future research. It is currently not known what the safety implications of chronic exposure to glyphosate are, even at low doses.

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SUMMARY

South Africa is considered a major GM crop producing country. The predominant GM trait in maize and soybean in South Africa is herbicide tolerance. Herbicide tolerant (HT) crops allow for the application of herbicide during the growing season to selectively kill weeds without damaging the crop. Glyphosate is the most widely used herbicide on HT crops in South Africa and the world.

Glyphosate is absorbed by HT crops after application. Studies have detected levels of up to 2.2 mg/kg in HT maize and 26 mg/kg in HT soybean. Glyphosate is not removed from grain by washing, cooking or processing. As a result of this, glyphosate can also be detected in processed food products. It has also been found that glyphosate can be detected in animal tissue and urine after exposure to the herbicide through feed. Similarly, glyphosate has also been detected in the urine of humans, either as a result of occupational exposure, through diet and/or water.

Pure glyphosate is considered safe by regulatory authorities and international bodies. However, recent studies have found that glyphosate in formulation at low concentrations results in endocrine disruption as well as DNA and chromosomal damage. As a result of this, the IARC re-classified glyphosate as a “probable human carcinogen”. Glyphosate is used on HT maize, a major staple food, and HT soybean, an important source of protein, in South Africa. Thus, the aim of this study was to determine whether glyphosate is present in commercially available South African food products containing maize and/or soybean as a primary constituent.

The majority of food products tested in this study contained glyphosate. The level of glyphosate ranged from 0.027 mg/kg to 2.257 mg/kg that is below the MRL and ADI established in South Africa. However, recent studies have shown that glyphosate in formulation is genotoxic at the levels found in maize and soybean containing foods in South Africa. The results from this study found that the level of glyphosate in food products in South Africa is comparable to the limited number of studies from the UK and USA. This study is unique to other published studies since it focused on food

products likely to be consumed daily. This study has confirmed that South Africans are exposed daily to low levels of glyphosate through food products.

The GM HT events in the food products were quantified in order to explain the variation in the levels of glyphosate. It was determined that 57 products contained one or more GM HT event in a range of 0.25% to 100%. However, there was no correlation between the level of glyphosate and percentage GM HT event in the products, even when GM HT negative samples were excluded from the analysis. This suggests that either glyphosate is not applied to some GM HT crops, when weed control is not required, or that the herbicide is applied to non-GM crops as a desiccant prior to harvesting.

Most of the food products used in this study were labelled in terms of GM content. The percentage GM HT event(s) in the food products was used to determine compliance to mandatory GM labelling in South Africa since the data was available. Results indicated that the majority of companies in South Africa are compliant with the Consumer Protection Act (2008) concerning GM labelling. However, most of the products labelled "GMO free", did not comply with the expectation of discerning consumers.

To conclude, this is the first study to investigate the extent of glyphosate in South African food products. This study has confirmed that South African consumers are exposed to low levels of glyphosate as a result of consuming maize and soybean food products. The level of glyphosate detected in the food products although considered low, is at a concentration reported to cause genotoxic effects at cellular level by *in vitro* studies. It is currently not known what the safety implications are of chronic exposure to glyphosate, through the consumption of a staple like maize meal and soybean in South Africa. The question of whether glyphosate in food is safe in the long term needs to be addressed through future research.

Keywords: Glyphosate, ELISA, GM crops, GM HT event detection, food products, GM labelling, herbicide tolerance, South Africa

OPSOMMING

Suid-Afrika is werêldwyd bekend as die negende grootste produsent van GM gewasse en verbou hoofsaaklik GM mielies, sojabone en katoen. Onkruidoder toleransie is die grootste GM eienskap in Suid-Afrika en kom voor in ongeveer 75% van alle GM gewasse wat geplant word. Onkruidoder tolerante (OT) gewasse laat die direkte toediening van onkruidoder toe, sonder om skade aan die gewas aan te rig. Die gewildste onkruidoder in Suid-Afrika asook werêldwyd is glifosaat en word hoofsaaklik gebruik vir toediening op OT gewasse.

Verskeie studies het getoon dat glifosaat geabsorbeer word deur OT gewasse na toediening. Glifosaat vlakke van tot 2.2 mg/kg is waargeneem in OT mielies en tot 26 mg/kg in OT sojabone. Glifosaat kan nie verwyder word van graan deur dit te was, gaar te maak of te proses nie. Die resultaat hiervan is dat glifosaat gevind word in geprosesseerde voedsel produkte. Verskeie studies het aangedui dat glifosaat geabsorbeer word en voorkom in die weefsel, uriene en fesus van diere wat gevoer is met glifosaat behandelde grane. Glifosaat is ook gevind in menslike uriene, hoofsaaklik as gevolg van kontak met die onkruidoder tydens landbou praktyk of deur die inname van glifosaat bevattende voedsel en water.

Verskeie internasionale owerhede beskou suiwer glifosaat as veilig vir mense en diere. Onlangse studies het egter bevind dat glifosaat in formulاسie toksies is teen lae konsentrasies. Die bevindinge sluit endokriene ontwrigting asook DNA en chromosomale skade in wat voorgekom het in beide mens en dierselle. In 2015 het die Internasionale Agentskap vir Navorsing oor Kanker (IARC) glifosaat geherklassifiseer as “waarskynlik karsinogenies vir mense”. OT mielies in die vorm van meliemeel is ‘n stapelvoedsel in Suid-Afrika en OT sojabone is n belangrike bron van proteïene. Beide hierdie gewasse word ekstensief behandel met glifosaat gedurende die groeiseisoen. Die doel van hierdie studie was dus, om te bepaal of glifosaat voorkom in kommersieel beskikbare Suid-Afrikaanse voedsel produkte, wat mielies of sojabone as die primêre bestanddeel bevat.

Glifosaat kom voor in die meerderheid van voedsel produkte getoets in hierdie studie met vlakke wat wissel van 0.027 mg/kg tot 2.257 mg/kg. Die vlakke van glifosaat in

die voedsel produkte was onder die toegelate limiete (MRL en ADI) vasgetel vir hierdie onkruidoder in Suid-Afrika. Onlangse studies dui egter aan dat glifosaat in formulasie toksies is teen konsentrasies gelykstaande aan wat voorkom in Suid-Afrikaanse mielie en sojaboon voedsel produkte. Die resultate van hierdie studie dui aan dat die konsentrasies glifosaat gevind in Suid-Afrikaanse voedsel produkte vergelykbaar is met die vlakke gevind in voedsel produkte van die Verenigde Koninkryk (VK) en die Verenigde State van Amerika (VSA). Hierdie studie is egter uniek omdat dit gefokus het op stapelvoedsel produkte wat daagliks geëet word. Die studie bevestig dat Suid-Afrikaners daagliks blootgestel word aan lae vlakke glifosaat deur hulle dieet.

Reële-tyd PKR was gebruik om die persentasie OT geen te bepaal wat voorkom in elke produk. Hierdie resultate het aangedui dat 57 uit die 81 produkte 'n OT geen bevat met vlakke wat wissel van 0.25% tot 100%. Geen korrelasie is egter gevind tussen die persentasie OT geen en die vlak van glifosaat in die produkte nie. 'n Verdere ondersoek is gedoen na al die produkte wat negatief getoets het vir n OT geen verwyder is, maar steeds is geen korrelasie gevind nie. Die bevindinge stel voor dat glifosaat nie noodwendig toegedien word tot alle OT gewasse nie. Dit kan verduidelik hoekom sommige voedsel produkte positief was vir n GM OT geen, maar geen glifosaat bevat nie. Dit word beskou as algemene landbou praktyk om nie-OT gewasse te spuit met glifosaat, om hierdie gewasse egalig uit te droog voor dit geoes word. Die bogenoemde verduidelik hoekom sommige produkte glifosaat bevat maar vry is van 'n GM OT geen.

Die voedsel produkte was ook ondersoek in terme van GM etikettering aangesien die persentasie GM OT geen per produk reeds bepaal was en meeste produkte 'n GM etiket bevat het. Die resultate van hierdie studie bevestig dat die meerderheid van maatskappye wat GM bevattende produkte produseer, voldoen aan die Wet op Verbruikersbeskerming ingestel ten opsigte van GM etikettering in Suid-Afrika. Die resultate dui egter aan dat sommige produkte gemerk "GMO vry", wel GM OT gene bevat en dus kontrasteer met wat hul etiket aandui. As gevolg hiervan mag die "GMO vry" handelsmerk verwarring skep, spesifiek by individue wat nie-GM produkte verkies.

Per opsomming, hierdie is die eerste studie wat ondersoek ingestel het ten opsigte van die voorkoms van glifosaat in die Suid-Afrikaanse voedselketting. Die studie het

bewys dat Suid Afrikaners daaglik bloot gestel word aan lae konsentrasies glifosaat deur die inname van mielie en sojaboon voedsel produkte. Onlangse studies het egter bevind, dat glifosaat in formulasie by soortgelyke konsentrasies soos gevind in die voedsel produkte getoets in hierdie studie, toksies is vir beide mens en dier. Dit is tans onbekend wat die gesondheids implikasies mag wees van kroniese blootstelling tot 'n lae konsentrasie glifosaat en moet dus in toekomstige studies ondersoek word.

Sluitelwoorde: Glifosaat, ELISA, GM gewasse, voedsel produkte, GM etikettering, onkruidodder toleransie, Suid-Afrika

APPENDIX A

A summary of the detected glyphosate level, standard deviation and limit of quantification of all products tested.

Sample identification number	Sample description	Mean of Glyphosate detected (ppb)	Standard Deviation (ppb)
1	Maize meal	46.52	0.55
2	Maize meal	82.91	2.52
3	Instant maize meal	34.58	0.74
4	Maize meal	41.99	1.48
5	Maize meal	59.71	4.55
6	Instant maize meal	49.83	3.67
7	Maize meal	47.81	2.96
8	Maize meal	43.96	2.92
9	Maize meal	81.98	0.59
10	Maize meal	26.90	2.94
11	Maize meal	33.85	1.91
12	Maize meal	43.85	3.52
13	Maize meal	91.64	3.29
14	Maize meal	32.71	0.94
15	Maize meal	63.39	3.98
16	Maize meal	45.63	5.61
17	Maize meal	68.06	5.35
18	Maize meal	92.83	5.26
19	Maize meal	48.27	1.10
20	Maize meal	35.47	0.89
21	Maize meal	43.86	8.55
22	Maize meal	34.33	3.89
23	Beer powder	94.79	3.38
24	Beer powder	40.19	0.18

(continued)

Sample identification number	Sample description	Average of Glyphosate detected (ppb)	Standard deviation (ppb)
25	Maize grits	ND	ND
26	Maize grits	ND	ND
27	Maize grits	ND	ND
28	Maize grits	ND	ND
29	Maize grits	ND	ND
30	Maize rice	40.98	0.98
31	Maize rice	28.34	2.61
32	Maize rice	65.42	4.59
33	Polenta	ND	ND
34	Polenta	ND	ND
35	Polenta	ND	ND
36	Polenta	ND	ND
37	Polenta	ND	ND
38	Corn flakes	ND	ND
39	Corn flakes	ND	ND
40	Corn flakes	ND	ND
41	Corn flakes	ND	ND
42	Corn flakes	ND	ND
43	Corn flakes	ND	ND
44	Corn flakes	ND	ND
45	Corn chips	ND	ND
46	Corn chips	ND	ND
47	Corn chips	ND	ND
48	Corn chips	ND	ND
49	Corn chips	ND	ND
50	Corn chips	ND	ND

(continued)

Sample identification number	Sample description	Average of Glyphosate detected (ppb)	Standard Deviation (ppb)
51	Corn chips	ND	ND
52	Corn chips	ND	ND
53	Corn chips	ND	ND
54	Corn chips	ND	ND
55	Maize Pasta	61.65	6.10
56	Maize Pasta	46.76	3.92
57	Maize Pasta	50.33	5.91
58	Corn-soy blend	59.34	5.94
59	Corn-soy blend	43.76	1.18
60	Corn-soy blend	52.73	4.92
61	Corn-soy blend	61.36	1.52
62	Corn-soy blend	64.54	10.57
63	Corn-soy blend	43.12	2.07
64	Soybean	48.92	1.25
65	Soy milk	142.18	8.98
66	Soy milk	39.75	0.68
67	Soy milk	35.69	4.38
68	Soy milk	31.71	3.96
69	Soy milk	51.84	2.48
70	Soy milk	49.25	12.45
71	Soy milk	81.20	4.27
72	Soy milk	50.37	7.89
73	Infants milk	57.88	5.64
74	Soy flour	27.14	1.03
75	Texturized soy protein	40.60	4.19
76	Texturized soy protein	921.33	3.83
77	Texturized soy protein	2257.04	53.02

(continued)

Sample identification number	Sample description	Average of glyphosate detected (ppb)	Standard deviation (ppb)
78	Texturized soy protein	78.11	5.72
79	Texturized soy protein	36.94	0.28
80	Texturized soy protein	1977.67	77.05
81	Texturized soy protein	195.07	12.77

ND: Not detected

APPENDIX B

A summary of the detected HT events, standard deviation and limit of quantification for the maize products.

Sample identification number	Sample description	HT event NK603 detected	Percentage standard deviation	Limit of quantification ¹	HT event GA21 detected	Percentage standard deviation	Limit of quantification
1	Maize meal	92.34%	6.26%	0.05%	Below LOQ	Below LOQ	0.07%
2	Maize meal	100.00%	2.26%	0.06%	ND	ND	ND
3	Instant maize meal	45.78%	4.41%	1.34%	Below LOQ	Below LOQ	1.41%
4	Maize meal	61.60%	1.10%	0.06%	ND	ND	ND
5	Maize meal	100.00%	3.73%	0.05%	Below LOQ	Below LOQ	0.07%
6	Instant maize meal	ND	ND	ND	ND	ND	ND
7	Maize meal	59.90%	5.51%	0.04%	ND	ND	ND
8	Maize meal	35.80%	0.87%	0.11%	ND	ND	ND
9	Maize meal	76.27%	5.56%	0.08%	Below LOQ	Below LOQ	0.09%
10	Maize meal	32.17%	0.82%	0.06%	ND	ND	ND
11	Maize meal	ND	ND	ND	ND	ND	ND

Maize products (continued)

Sample identification number	Sample description	HT event NK603 detected	Percentage standard deviation	Limit of quantification	HT event GA21 detected	Percentage standard deviation	Limit of quantification
12	Maize meal	1.28%	0.01%	0.03%	Below LOQ	Below LOQ	0.07%
13	Maize meal	32.55%	0.58%	0.07%	ND	ND	ND
14	Maize meal	30.45%	1.51%	0.08%	ND	ND	ND
15	Maize meal	6.44%	0.52%	0.11%	ND	ND	ND
16	Maize meal	35.97%	1.48%	0.05%	ND	ND	ND
17	Maize meal	4.77%	0.69%	0.03%	Below LOQ	Below LOQ	0.05%
18	Maize meal	24.95%	0.48%	0.05%	ND	ND	ND
19	Maize meal	22.81%	1.37%	0.07%	ND	ND	ND
20	Maize meal	80.61%	3.14%	0.83%	ND	ND	ND
21	Maize meal	27.07%	1.88%	0.03%	0.72%	0.03%	0.09%
22	Maize meal	26.62%	1.48%	0.03%	ND	ND	ND
23	Beer powder	26.24%	0.63%	7.62%	ND	ND	ND

Maize products (continued)

Sample identification number	Sample description	HT event NK603 detected	Percentage standard deviation	Limit of quantification	HT event GA21 detected	Percentage standard deviation	Limit of quantification
24	Beer powder	40.47%	0.71%	0.02%	ND	ND	ND
25	Maize grits	54.63%	3.35%	0.03%	ND	ND	ND
26	Maize grits	42.51%	2.31%	0.03%	Below LOQ	Below LOQ	0.16%
27	Maize grits	55.18%	1.04%	0.07%	Below LOQ	Below LOQ	0.16%
28	Maize grits	16.39%	1.02%	0.05%	Below LOQ	Below LOQ	0.11%
29	Maize grits	21.50%	2.01%	0.06%	Below LOQ	Below LOQ	0.16%
30	Maize rice	67.47%	1.79%	0.07%	ND	ND	ND
31	Maize rice	49.41%	0.99%	0.08%	ND	ND	ND
32	Maize rice	9.72%	0.71%	0.03%	Below LOQ	Below LOQ	0.09%
33	Polenta	0.25%	0.01%	0.03%	ND	ND	ND
34	Polenta	53.65%	0.34%	0.03%	ND	ND	ND
35	Polenta	11.55%	0.39%	0.04%	ND	ND	ND

Maize products (continued)

Sample identification number	Sample description	HT event NK603 detected	Percentage standard deviation	Limit of quantification	HT event GA21 detected	Percentage standard deviation	Limit of quantification
36	Polenta	31.56%	0.30%	0.03%	ND	ND	ND
37	Polenta	ND	ND	ND	ND	ND	ND
38	Corn flakes	ND	ND	ND	ND	ND	ND
39	Corn flakes	ND	ND	ND	ND	ND	ND
40	Corn flakes	ND	ND	ND	ND	ND	ND
41	Corn flakes	ND	ND	ND	ND	ND	ND
42	Corn flakes	ND	ND	ND	ND	ND	ND
43	Corn flakes	ND	ND	ND	ND	ND	ND
44	Corn flakes	ND	ND	ND	ND	ND	ND
45	Corn chips	11.54%	0.83%	0.05%	ND	ND	ND
46	Corn chips	41.65%	3.69%	0.02%	ND	ND	ND
47	Corn chips	33.15%	2.23%	0.05%	ND	ND	ND
48	Corn chips	35.33%	0.97%	0.05%	ND	ND	ND

Maize products (continued)

Sample identification number	Sample description	HT event NK603 detected	Percentage standard deviation	Limit of quantification	HT event GA21 detected	Percentage standard deviation	Limit of quantification
49	Corn chips	10.76%	1.68%	0.05%	ND	ND	ND
50	Corn chips	20.36%	2.82%	0.08%	ND	ND	ND
51	Corn chips	17.54%	0.51%	0.05%	ND	ND	ND
52	Corn chips	16.35%	0.62%	0.05%	ND	ND	ND
53	Corn chips	12.22%	0.27%	0.07%	ND	ND	ND
54	Corn chips	ND	ND	ND	ND	ND	ND
55	Maize pasta	21.15%	1.11%	0.02%	ND	ND	ND
56	Maize pasta	ND	ND	ND	ND	ND	ND
57	Maize pasta	ND	ND	ND	ND	ND	ND

ND: Not detected

1 The limit of quantification is based on the copy number of the reference gene *HMG* in maize and *Lectin* in soybean

A summary of the detected HT events, standard deviation and limit of quantification for the corn-soy blends.

Sample identification number	Sample description	HT event NK603 detected	Percentage standard deviation	Limit of quantification ¹	HT event GA21 detected	Percentage standard deviation	Limit of quantification	HT event GTS40-3-2 detected	Percentage standard deviation	Limit of quantification
58	Corn-soy blend	0.04%	0.01	0.04%	ND	ND	ND	ND	ND	ND
59	Corn-soy blend	0.12%	0.01%	0.08%	ND	ND	ND	Below LOQ	Below LOQ	0.07%
60	Corn-soy blend	ND	ND	ND	ND	ND	ND	0.14%	0.01%	0.07%
61	Corn-soy blend	ND	ND	ND	ND	ND	ND	ND	ND	ND
62	Corn-soy blend	16.70%	0.28%	0.02%	ND	ND	ND	48.65%	1.73%	0.40%
63	Corn-soy blend	ND	ND	ND	ND	ND	ND	4.01%	0.11%	0.07%

ND: Not detected

¹ Limit of quantification is based on the copy number of the reference gene *HGM* in maize and *Lectin* in soybean

A summary of the detected HT events, standard deviation and limit of quantification for the soybean products.

Sample identification number	Sample description	HT event GTS40-3-2 detected	Percentage standard deviation	Limit of quantification ¹
64	Soybean	ND	ND	ND
65	Soy milk	9.57%	0.76%	0.07%
66	Soy milk	Below LOQ	Below LOQ	0.07%
67	Soy milk	ND	ND	ND
68	Soy milk	Below LOQ	Below LOQ	0.05%
69	Soy milk	ND	ND	ND
70	Soy milk	ND	ND	ND
71	Soy milk	5.21%	1.31%	0.10%
72	Soy milk	ND	ND	ND
73	Infants milk	0.07%	0.01%	0.05%
74	Soy flour	ND	ND	ND

ND: Not detected

¹ Limit of quantification is based on the copy number of the reference gene *HMG* for maize and *Lectin* for soybean

A summary of the detected HT events, standard deviation and limit of quantification for the texturized soy protein products.

Sample identification number	Sample description	HT event NK603 detected	Percentage standard deviation	Limit of quantification ¹	HT event GA21 detected	Percentage standard deviation	Limit of quantification	HT event GTS40-3-2 detected	Percentage standard deviation	Limit of quantification
75	Texturized soy protein	ND	ND	ND	ND	ND	ND	ND	ND	ND
76	Texturized soy protein	27.75%	1.00%	0.33%	ND	ND	ND	84.70%	2.02%	1.61%
77	Texturized soy protein	43.22%	2.22%	0.19%	ND	ND	ND	92.09%	1.26%	1.09%
78	Texturized soy protein	ND	ND	ND	ND	ND	ND	16.75%	0.32%	0.12%
79	Texturized soy protein	ND	ND	ND	ND	ND	ND	Below LOQ	Below LOQ	0.06%
80	Texturized soy protein	ND	ND	ND	ND	ND	ND	78.10%	3.16%	0.01%
81	Texturized soy protein	54.38%	2.19%	0.27%	ND	ND	ND	56.55%	2.38%	6.88%

ND: Not detected

¹ Limit of quantification is based on the copy number of the reference gene *HMG* for maize and *Lectin* for soybean

APPENDIX C

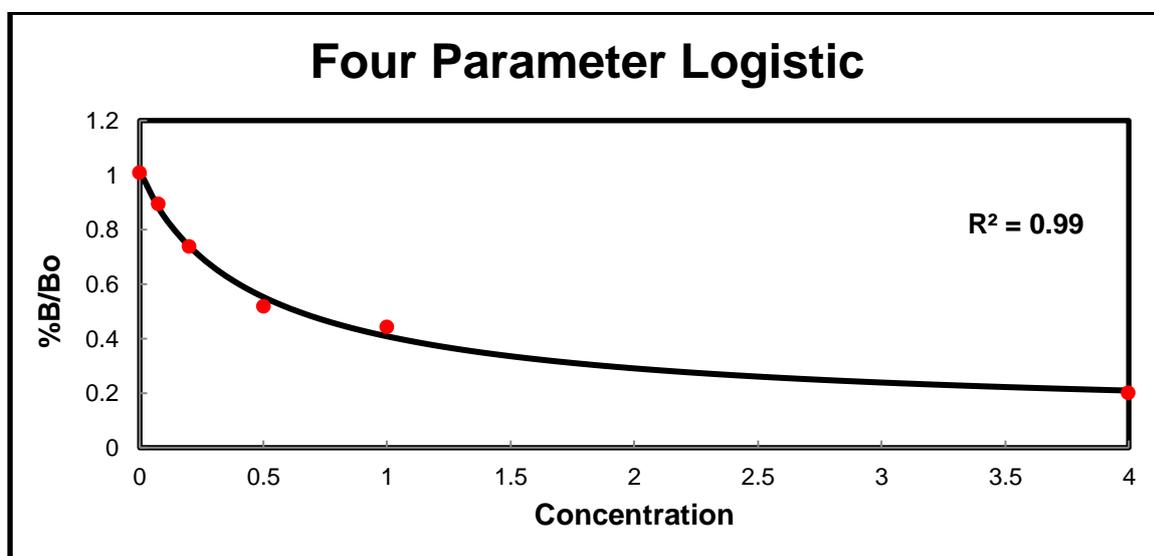


Figure A: Example of the Four Parameter Logistic curve generated to determine the glyphosate concentration of the maize and soybean food products in parts per billion (ppb). The %B/B₀ was calculated for the standards by dividing the mean absorbance value of each the standard by the mean absorbance value of the zero standard. The %B/B₀ calculated for the five standards (red) were used to generate a Four Parameter Logistic curve with a R² of > 0.98. The %B/B₀ calculated for each sample (mean absorbance of sample/mean absorbance of zero standard) was plotted on the Four Parameter Logistic curve to determine the glyphosate concentration present in the samples in ppb.

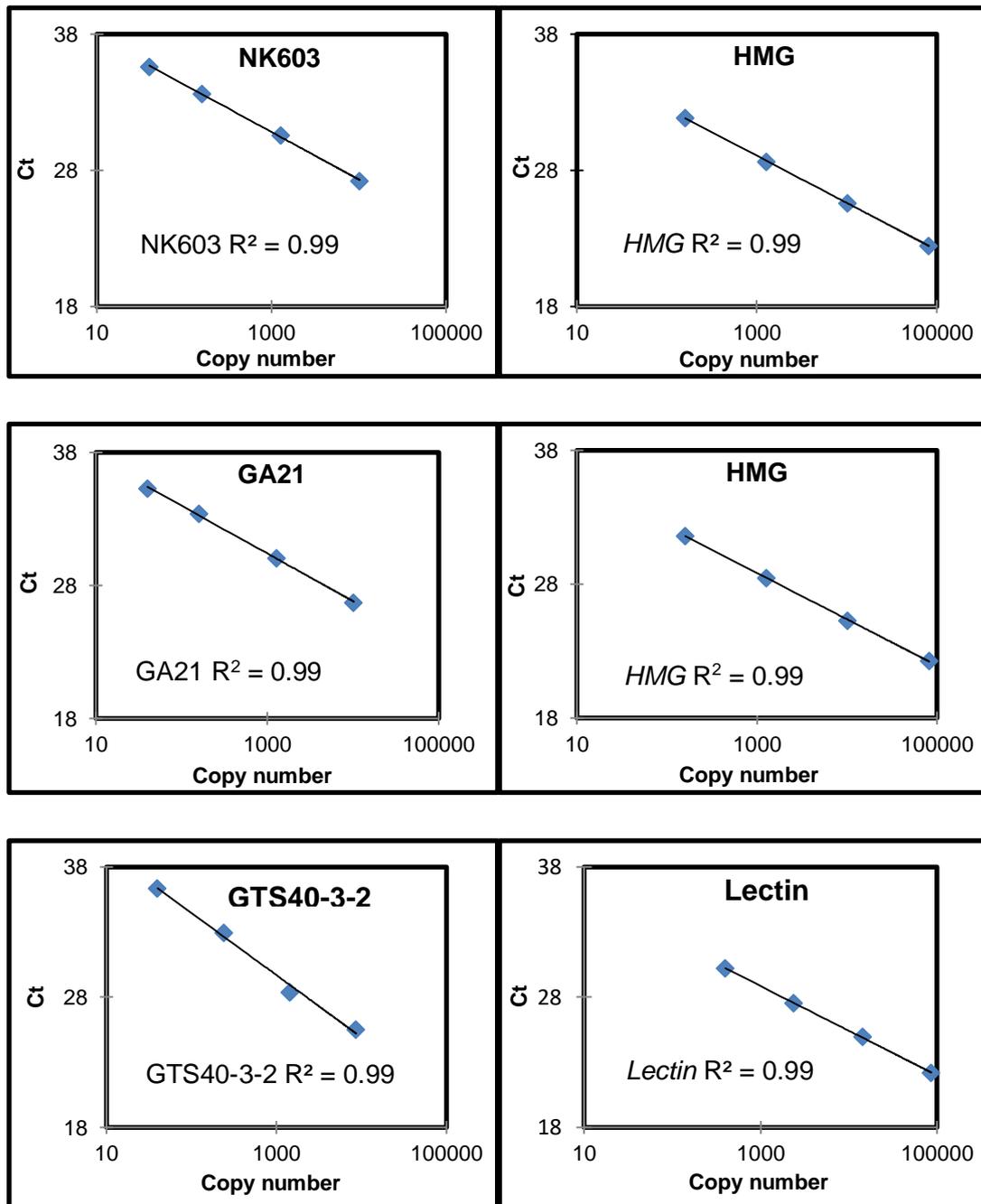


Figure B: Example of the standard curve generated during quantitative Real-time PCR to determine the percentage of GM HT event NK603, GA21 and GTS40-3-2 in maize and/or soybean products. Four copy number standards (blue) in duplicate were used to generate a standard curve with a R^2 of > 0.98 .