Agrochemicals related to maize crops in South Africa and associated biological effects: an *in vitro* study

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ABSTRACT

Although agrochemicals reduce pest-associated crop losses, these chemicals end up in non-target environments following rainfall and irrigation. When fractions of agrochemical mixtures are bioavailable, they pose a threat to human and environmental health. The aim of this study was to determine whether the water-soluble fraction of agricultural soils in South Africa elicit selected biological effects *in vitro*, as well as to determine which pesticides are likely causing the observed effects. Composite soil samples were collected in two maize growing regions—the Mpumalanga province and Vaalharts Valley—known for their extensive herbicide application. Water-soluble compounds were extracted from the soil using deionised water to mimic environmental conditions and obtain the bioavailable fraction. The H4IIE-*luc* reporter gene bioassay was performed to establish whether the soil-extracts contained aryl hydrocarbon receptor (AhR) ligands. This was followed by assessing (anti-)androgenic and glucocorticoid activity using the human breast carcinoma cell line MDA-kb2. The T47D-KB*luc* cell line was used to screen the water-soluble agrochemical residues for (anti-)oestrogenicity by evaluating binding to the oestrogen receptor. Oxidative stress responses (reactive oxygen species production, superoxide dismutase content, catalase activity, and lipid peroxidation), and non-neuronal acetylcholinesterase activity was also evaluated *in vitro* using the rat hepatoma H4IIE-*luc* and human duodenum HuTu-80 cell lines. Lastly, ultra-high performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry was used to quantify the following current-use pesticides in the soil: 2,4-dichlorophenoxy acetic acid (2,4-D), atrazine, dicamba, and imidacloprid. Results from the *in vitro* bioassays indicated that compounds present in the samples did not activate the AhR or androgen receptor (AR). However, at the concentrations evaluated in this study the soil-extracts from some sampling locations caused AR antagonism and (anti-)oestrogenicity, indicating endocrine disruptive effects. Although similar responses were observed in the HuTu-80 and H4IIE-*luc* cells following exposure to the bioavailable fraction for 24 hours (83 mg/mL), the rat hepatoma cell line was able to detoxify the xenobiotics present in the samples better. Furthermore, all four the target pesticides were quantifiable in at least one of the soil samples. Atrazine (89%) had the highest detection frequency, followed by dicamba (84%), 2,4-D (74%), and imidacloprid (32%). The combined use of *in vitro* bioassays and instrumental chemical analysis provided a holistic overview of the impact of agriculture on non-target organisms in the South African environment. Moreover, the findings of the present study contribute to the current understanding of the biological effects associated with the water-soluble fraction of agricultural soils, as well as the identity of the current-use pesticides likely causing these effects.

Keywords: Endocrine disruption; mammalian tissue culture; oxidative stress; pesticides; soil.

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LIST OF ABBREVIATIONS

%TOC	Percentage total organic carbon
2,4-D	2,4-Dichlorophenoxyacetic acid

A

ABS_{NC}	Mean absorbance of negative control
ABS_{S}	Absorbance of cells exposed to the sample
ABS_{SC}	Mean absorbance of the solvent control
ACh	Acetylcholine
AChE	Acetylcholinesterase
AChR	Acetylcholine receptor
AhR	Aryl hydrocarbon receptor
AR	Androgen receptor
ARNT	Aryl hydrocarbon receptor nuclear translocator
ASE	Accelerated solvent extraction
ATP	Adenosine-5'-triphosphate

B

BC(s)	Blank control(s)
BChE	Butyrylcholinesterase
BSA	Bovine serum albumin

C

$CaCl_2$	Calcium chloride
CAT	Catalase
CDT	Charcoal dextran treated
$CH_3COOH_{(c)}$	Glacial acetic acid
CO_2	Carbon dioxide
CYP450	Cytochrome P450

D

DCF	2'-7'Dichlorofluorescein
DCFH	2'-7'Dichlorodihydrofluorescein
Dexamethasone-EQs	Dexamethasone equivalents
DHT	5α-Dihydrotestosterone
DMEM	Dulbecco's Modified Eagle's Medium

DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
DPBS	Dulbecco's phosphate-buffered saline
DST	Department of Science and Technology
DTNB	5,5'-Dithio-*bis*-(2-nitrobenzoic acid)
DTPA	Diethylenetriaminepentaacetic acid
DTT	Dithiolthreitol

E

E_2	17β-Oestradiol
EBM(s)	Effects-based method(s)
EC	Effects concentration
EC_{20}	Effects concentration producing 20% effect (agonism)
EC_{50}	Effects concentration producing 50% effect (agonism)
EC_{80}	Effects concentration producing 80% effect (agonism)
EDC(s)	Endocrine disrupting chemical(s)
EDTA	Ethylenediaminetetraacetic acid
EE_2	17α-Ethinyl oestradiol
E_2-EQs	17β-Oestradiol equivalents
EQ(s)	Equivalent(s)
ER	Oestrogen receptor
ERE	Oestrogen response element
ESI	Electrospray ionisation

F

FBS	Foetal bovine serum
FC	Fold change
Flutamide-EQs	Flutamide equivalents

G

GIT	Gastrointestinal tract
GR	Glucocorticoid receptor
GSH	Reduced form of glutathione
GSSG	Oxidised form of glutathione (glutathione disulphide)

H

HAHs	Halogenated aromatic hydrocarbons
HCl	Hydrochloric acid

H_2DCF-DA	2'-7'Dichlorodihydrofluorescein diacetate
HDPE	High-density polyethylene
HEPA	High efficiency particulate air
HEPES	4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid
HNE	4-Hydroxynonenal
H_2O	Water
H_2O_2	Hydrogen peroxide
HRE	Hormone response element
H_2SO_4	Sulphuric acid

I

IC	Inhibitory concentration
IC_{20}	Inhibitory concentration producing 20% effect (antagonism)
IC_{50}	Inhibitory concentration producing 50% effect (antagonism)
IC_{80}	Inhibitory concentration producing 80% effect (antagonism)
ICI	Fulvestrant
ICI-EQs	ICI equivalents
ISO	International Organisation for Standardisation

K

K_2HPO_4	Dipotassium hydrogen phosphate
KH_2PO_4	Potassium dihydrogen phosphate
$KMnO_4$	Potassium permanganate

L

L-15	Leibovitz
LAR	Luciferase assay reagent
LOD	Limit of detection
LOQ	Limit of quantification
LPO	Lipid peroxidation

M

M1–M15	Maize field 1-Maize field 15
MDA	Malondialdehyde
$(MgCO_3)_4Mg(OH)_2.5H_2O$	Magnesium carbonate hydroxide pentahydrate
$MgSO_4.7H_2O$	Magnesium sulphate heptahydrate
MMTV	Mouse mammary tumour virus
MP	Mpumalanga province

mRNA	Messenger ribonucleic acid
MS	Mass spectrometer
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
m/z	Mass-to-charge ratio

N

n	Number of pairs
NADH	Nicotinamide adenine dinucleotide + hydrogen
$NaHCO_3$	Sodium bicarbonate
NC	Negative control
NNCS	Non-neuronal cholinergic system
NRF	National Research Foundation
NWU	North-West University

O

O_2	Molecular oxygen
$O_2^{\bullet-}$	Superoxide radical
OCPs	Organochlorine pesticides
$^{\bullet}OH$	Hydroxyl radical
OP	Organophosphate

P

P1–P4	Pecan orchard 1–Pecan orchard 4
PAHs	Polyaromatic hydrocarbons
PC(s)	Positive control(s)
PCBs	Polychlorinated biphenyls
PCDL	Personal Compound Database and Library
POPs	Persistent organic pollutants

Q

QC	Quality control
Q-TOF	Quadrupole time-of-flight
QuEChERS	Quick, easy, cheap, effective, rugged, safe

R

R^2	Coefficients of determination
RFU(s)	Relative fluorescence unit(s)
RLU(s)	Relative light unit(s)

RNA	Ribonucleic acid
ROS	Reactive oxygen species
RPMI-1640	Roswell Park Memorial Institute-1640
r_s	Spearman's rho coefficient
RSD	Relative standard deviation

S

SC(s)	Solvent control(s)
SDS	Sodium dodecyl sulphate
SOD	Superoxide dismutase
SPSS	Statistical Package for Social Sciences

T

TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
TCDD	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin
TCDD-EQs	TCDD equivalents
Testosterone-EQs	Testosterone equivalents
TMP	1,1,3,3-Tetramethoxypropane
TNB	2-Nitro-5-thiobenzoate
TOC	Total organic carbon
TOF	Time-of-flight

U

UHPLC-QTOF/MS	Ultra-high performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry
UV	Ultraviolet

V

VH	Vaalharts Valley

W

WHP	Weighted hazard potential
WRC	Water Research Commission
WWTPs	Wastewater treatment plants

X

XRE	Xenobiotic response element

LIST OF MEASURING UNITS

%	Percentage
°C	Degrees Celsius
cells/mL	Cells per milliliter
cm^2	Square centimeter
g	Gram(s)
g	Gravitational force
GHz	Gigahertz
g/L	Gram(s) per liter
kPa	Kilopascal
L/min	Liter(s) per minute
M	Molar
mg/mL	Milligram(s) per milliliter
mg/kg/day	Milligram(s) per kilogram per day
mL	Milliliter(s)
mL/minute	Milliliter(s) per minute
mm	Millimeter(s)
mM	Millimolar
MΩ-cm	Megaohm(s) per centimeter
ng/mL	Nanogram(s) per milliliter
ng SOD/mg protein	Nanograms superoxide dismutase per milligram protein
nm	Nanometer(s)
nM	Nanomolar
pg/g	Picogram(s) per gram
pg/mL	Picogram(s) per milliliter
pH	Potential of hydrogen
rpm	Revolutions per minute
µg/g	Microgram(s) per gram
µL	Microliter(s)
µL/well	Microliter(s) per well
µg/mL	Microgram(s) per milliliter
µm	Micrometer(s)
µM H_2O_2/min/mg protein	Micromolar hydrogen peroxide per minute per milligram protein
V	Volt(s)
v/v	Volume to volume
v/w	Volume to mass

CHAPTER 1: INTRODUCTION

1.1 General background

Agrochemicals, for example pesticides, have been widely applied in the agricultural sector to reduce crop losses. As a result, pesticides play a key role in feeding the global population (Mancini *et al*., 2019). In sub-Saharan Africa, South Africa is the largest user of pesticides (Dabrowski *et al*., 2014) with more than 500 pesticides already registered almost a decade ago (Quinn *et al*., 2011). In recent years the number has increased to over 3 000 (Dabrowski, 2015a), as pesticides are widely applied to a variety of crops. Maize is an important staple food and livestock fodder in South Africa and is also the crop that is sprayed with the most pesticides (Dabrowski *et al*., 2014). The authors determined this by expressing the weighted hazard potential (WHP) as a ratio between the total amount of pesticides applied to a specific crop and the total use of all pesticides applied in the country, with maize then having the highest WHP (Dabrowski *et al*., 2014).

1.2 Problem statement

Although many studies evaluate the single effects of the active ingredients of pesticides (Abdel-Halim & Osman, 2020; Huang *et al*., 2020a), these observations are not a true representation of the state of agrochemicals in the environment. The use of single chemical risk assessments is not adequate to estimate the actual risks posed by pesticide mixtures present in the environment. Moreover, commercial pesticides do not only contain the active compound but are complex formulations that contain chemical additives or "inert" compounds (i.e., co-formulants, adjuvants, surfactants) (Mesnage *et al*., 2019). The inert compounds constitute 90–95% of the commercial formulation and may be more toxic than the active ingredient itself (Md Meftaul *et al*., 2020). These additives increase the active compound's efficacy (Mesnage *et al*., 2019), solubility (Mesnage *et al*., 2014), and stability (Cox & Surgan, 2006; Mesnage & Antoniou, 2018). The designation of certain co-formulants as "inert" is worrisome as it exempts them from the requirement of establishing a tolerance limit and ultimately being subjected to chronic toxicity testing (Mesnage *et al*., 2019). Unfortunately, as co-formulants are not regulated in the same manner as the active ingredient, their toxic effects are generally overlooked (Mesnage & Antoniou, 2018).

Furthermore, in real life agricultural practices, pesticides, and other agrochemicals (e.g., fertilisers, fumigants, synthetic hormones, and seed coatings) are not used in isolation (Archer & Van Wyk, 2015; Tang & Maggi, 2021). Consequently, agrochemicals end up in the environment as complex mixtures, possibly eliciting combinatorial effects when bioavailable (Kortenkamp, 2008). In the environment, only a fraction of the total quantity of a xenobiotic is potentially available for uptake by living organisms. Bioavailable refers to the fraction of a chemical (including pesticides) that is freely available or accessible

to living organisms for uptake across cellular membranes (US NRC, 2003). Once this transfer across cellular membranes occurs, the fraction can be stored, transformed, assimilated, or degraded within the living organism (Semple *et al*., 2004).

The physico-chemical properties of pesticides govern their behaviour, persistence, and bioavailability in the environment (Gevao *et al*., 2000; Pathak *et al*., 2022). Pesticides with short half-lives are less persistent, while those with longer half-lives are highly persistent in soil (Raffa & Chiampo, 2021). Consequently, persistent chemicals are present for longer and leach through the soil over time (Mandal *et al*., 2020), reaching the groundwater level (Pathak *et al*., 2022). What is more, events such as irrigation or heavy rainfall can promote the movement of polar compounds from the soil to non-target aquatic environments (e.g., rivers, streams, or dams) (Mitra & Raghu, 1998; Mandal *et al*., 2020). For example, water-soluble formulations applied to plants are not very persistent in soil as they are washed away more readily by rainfall (Md Meftaul *et al*., 2020). Durães *et al*. (2018) state that highly hydrophobic pesticides readily adsorb to soil particles, while polar compounds are more mobile in soils. The size of the soil particles also play a role in the movement of chemicals through the soil environment. Fine soil particles, such as those in sandy soil with low organic content, present an unstable capturing system and pesticides will therefore leach more easily (Durães *et al*., 2018; Pathak *et al*., 2022).

Due to the migration of polar agrochemicals through soil (Durães *et al*., 2018), there is a need to determine the biological effects associated with specifically the water-soluble (i.e., bioavailable) fraction of environmental matrices. The current study is thus novel in the sense that it employed both cell-based *in vitro* bioassays and chemical analysis to investigate the effects and presence of polar compounds in agricultural soils from South Africa. By investigating the biological effects (endocrine disruption and oxidative stress) associated with environmental mixtures and quantifying the chemicals present, this study provides insights into the real-life threat that water-soluble, current-use agrochemicals may pose to humans and wildlife.

1.3 Hypothesis

Agricultural soils in South Africa contain quantifiable levels of water-soluble agrochemicals which induce biological responses *in vitro*.

1.4 Aims and objectives

Aim 1: Determine the endocrine disrupting potential of the bioavailable fraction of agricultural chemical residues related to maize crop and pecan nut farming.

Specific objectives:

- Extract the water-soluble fraction from the soil to be used in biological assays with deionised water (liquid-solid extraction) to mimic environmental conditions.

- Establish if the water-soluble compounds present in maize field and pecan orchard soils contain aryl hydrocarbon receptor (AhR) ligands using an *in vitro* H4IIE-*luc* reporter gene bioassay.
- Determine androgen receptor (AR) and glucocorticoid receptor (GR) activity of the same water-soluble fraction by performing an *in vitro* cell-based MDA-kb2 reporter gene bioassay.
- Assess the (anti-)oestrogenicity of the polar compounds in soils from maize fields and pecan orchards *in vitro* using the T47D-KB*luc* reporter gene bioassay.

Aim 2: Measure the oxidative stress potential and resulting oxidative damage of water-soluble agrochemicals present in agricultural soils.

Specific objectives:

- Evaluate whether the polar compounds present in the maize field and pecan orchard soils caused reactive oxygen species (ROS) production in intestinal (HuTu-80) and liver (H4IIE-*luc*) cells using the 2'-7'dichlorodihydrofluorescein diacetate (H_2DCF-DA) bioassay.
- Investigate the effect of the above-mentioned water-soluble compounds on the first line of defence antioxidant enzymes by measuring superoxide dismutase (SOD) content and catalase (CAT) activity *in vitro*.
- Determine if the maize field and pecan orchard soils contain compounds that interact with the lipid components in the cellular membranes of HuTu-80 and H4IIE-*luc* cells by means of the thiobarbituric acid reactive substances (TBARS) assay.

Aim 3: Investigate whether the water-soluble compounds of maize field and pecan orchard soils interfere with the non-neuronal cholinergic system (NNCS).

Specific objective:

- Measure the production of non-neuronal acetylcholinesterase (AChE) in the HuTu-80 and H4IIE-*luc* cell lines by performing Ellman's bioassay.

Aim 4: Identify the pesticides present in the bioavailable fraction of soils associated with maize crops.

Specific objective:

- Perform instrumental chemical analysis of the water-soluble fraction of the maize and pecan soil extracts using ultra-high performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (UHPLC-QTOF/MS) to quantify four selected pesticides (i.e., 2,4-dichlorophenoxyacetic acid, atrazine, dicamba, and imidacloprid) and screen for other agrochemicals.

1.5 Structure of thesis

This thesis represents a compilation of six chapters written according to the format prescribed by the NWU to comply with the university's requirements for the fulfillment of the degree Doctor of Philosophy in Science with Environmental Sciences.

Chapter 1 is the current chapter and serves as a brief introduction to the study. This chapter also includes the problem statement, hypothesis, aims and specific objectives of the study, as well as the structure of the thesis and author contributions of the published manuscript originating from this study.

Chapter 2 comprises an extensive review of current literature, including maize production and the use of agrochemicals in South Africa, selected biological effects (i.e., xenobiotic metabolism, endocrine disruption, oxidative stress, acetylcholinesterase inhibition) associated with agrochemical exposure, and the use of effects-based methods and chemical analysis to quantify these effects.

Chapter 3 provides a concise description of the materials used and methods undertaken during this study.

Chapter 4 describes the results obtained pertaining to the soil properties, *in vitro* bioassays, and chemical analysis.

Chapter 5 presents the interpretation and in-depth discussion of the results reported in Chapter 4 and how they relate to current literature.

Chapter 6 provides a general conclusion of the study based on the results obtained. The major findings of the study are discussed. Limitations identified during the study and recommendations for future avenues of research are also included.

References are given at the end of the thesis to avoid repetition of certain references. The Harvard reference style (parenthetical referencing) as specified by the NWU was used.

Annexure A contains supplementary information for Chapter 3.
Annexure B contains supplementary information for Chapter 4.
Annexure C contains the title page of the published manuscript originating from this study.

As required by the A-rules of the NWU, author contributions for the published manuscript originating from this study are provided:

1. Horak, I., Horn, S. & Pieters, R. (2021). Agrochemicals in freshwater systems and their potential as endocrine disrupting chemicals: A South African context. *Environmental Pollution*, 268:115718. https://doi.org/10.1016/j.envpol.2020.115718

Author	Contribution	Consent
I. Horak	First author; contributed to the conceptualisation of the papers and was responsible for writing the original manuscript drafts.	
S.R. Horn	Co-promoter and second author; supervised all aspects of the study; contributed to the conceptualisation of the papers; was responsible for reviewing and editing the manuscripts; approved the submitted manuscripts; and acquired project funding.	
R. Pieters	Promoter and third author; supervised all aspects of the study; contributed to the conceptualisation of the papers; was responsible for reviewing and editing the manuscripts; approved the submitted manuscripts; and acquired project funding.	

CHAPTER 2: LITERATURE REVIEW

2.1 Maize production in South Africa

South Africa's climate is described as semi-arid with a mean annual temperature of 18°C (Jury, 2021; Meza *et al*., 2021). However, there are regions within the country where the climate ranges from Mediterranean in the southwest to subtropical on the eastern coast, and temperate in the northeast (Meza *et al*., 2021). South Africa has an average annual rainfall of ~464 mm, with more than 80% of the country's annual rainfall between November and March (Chikoore & Jury, 2021). However, the country's rainfall is considered variable with the northeast and southwest receiving austral winter rainfall (May to August), while the rest of the country relies on austral summer rainfall (September to February) (Zvarevashe *et al*., 2018). These climatic conditions make South Africa ideal for the cultivation of a large variety of crops. An estimated 37.9% of the country's 122.5 million hectares of land is used for commercial agriculture (Statistics SA, 2020). The majority of this land is used for livestock and game farming (36.5 million hectares), while 7.6 million hectares of arable land is used for the production of various field crops (maize, soybean, wheat, sunflower, sorghum, barley, and canola) and fruit farming (pome and stone fruit, table grapes, and nuts). South Africa is therefore highly dependent on the agriculture sector to feed the country's growing population of nearly 60 million (FAOSTAT, 2022a). Apart from ensuring food security, the country's agriculture sector also has important socio-economic implications, including job creation, poverty alleviation, rural development, and foreign exchange, with South Africa's agriculture sector contributing about 3% to the country's national gross domestic product (Meza *et al*., 2021).

Although a large variety of crops are cultivated, South Africa's agricultural sector is dominated by industrialised maize production in the eastern parts of the country (Chikoore & Jury, 2021). South Africa is among the top ten maize-producing countries globally (FAOSTAT, 2020a). Approximately 16.2 million metric tons of maize on 2.7 million hectares of land were produced during the 2020/2021 growing season. The Free-State (43.13%), Mpumalanga (23.92%) and North West (16.53%) provinces are the main maize-producing regions in South Africa, responsible for more than 80% of the country's total maize production (Grain SA, 2021). The country's surplus maize is also exported to our neighbouring countries (Botswana, Lesotho, Mozambique, Namibia, Swaziland, and Zimbabwe), but also to North and South Korea (Grain SA, 2017). In 2020, South Africa exported almost 2.6 million tons of surplus maize to the value of US$566 million (Nuss & Tanumihardjo, 2010; FAOSTAT, 2022b). Apart from the economic benefit of maize exportation, maize is also an important staple food and livestock fodder (Adisa *et al*., 2018; Chikoore & Jury, 2021) as it is rich in carbohydrates, vitamins A and E, and minerals such as phosphorus and zinc (Nuss & Tanumihardjo, 2010). Maize production is therefore essential in reducing hunger and food insecurity, not only in South Africa but globally.

2.2 The use of agrochemicals in South Africa

Unfortunately, maize crop plants are susceptible to various biotic and abiotic factors that affect maize production. Weeds are amongst the foremost biotic factors negatively influencing maize crop yield. Globally, 37% of maize crop yield losses are attributed to weeds which compete with the maize plants for water, sunlight, and essential nutrients. Chemical management is considered the most effective and low-cost method to control weeds (Sharma & Rayamajhi, 2022). Therefore, to maintain agricultural productivity and sustain South Africa's growing population, an extensive number of pesticides—especially herbicides—are applied (Quinn *et al*., 2011; FAOSTAT, 2020b). When these herbicides are directly sprayed onto weeds, up to 80% reach the target. However, if not directly applied (e.g., aerial application), only an estimated 0.1–5% of post-emergent herbicides applied to maize reach their target (i.e., weeds) (Pimentel & Levitan, 1986). Moreover, modern-day agricultural practices rely on the use of pesticide combinations (e.g., fungicides, herbicides, insecticides, and rodenticides) (Archer & Van Wyk, 2015; Tang & Maggi, 2021). Other agrochemicals, such as chemosterilants, synthetic hormones, plant growth regulators, synthetic fertilisers, and seed coatings are also used (Horak *et al*., 2021). Consequently, all these applied chemicals end up in the environment (i.e., atmosphere, soil, sediment, and water) as complex mixtures (Kortenkamp, 2008), contaminating ecosystems and causing unintended biological effects in non-target organisms (Pimentel, 1995). In South Africa, four of the most used pesticides include the three herbicides 2,4-dichlorophenoxyacetic acid (2,4-D), atrazine and dicamba, and the insecticide imidacloprid (Dabrowski *et al*., 2014).

2.2.1 2,4-Dichlorophenoxyacetic acid (2,4-D)

2,4-Dichlorophenoxyacetic acid is a pre- and post-emergent herbicide used to control broadleaf weeds (Islam *et al*., 2018) in agriculture, forestry, and lawn care (Harada *et al*., 2016; Gaaied *et al*., 2019). It was developed during the Second World War as one of the active ingredients of Agent Orange and was the first synthetic herbicide to be commercially sold with registered use dating back to 1946 (Song, 2014; Hattab *et al*., 2015; Islam *et al*., 2018). 2,4-Dichlorophenoxyacetic acid falls in the chemical class of chlorophenoxy herbicides which are physiologically similar to auxins—natural plant growth hormones (Ozcan Oruc *et al*., 2004). The mode of action of 2,4-D has been thoroughly reviewed by Song (2014). Briefly, 2,4-D mimics natural auxins at a molecular level which causes uncontrollable and disorganised plant growth. This causes the passage of nutrients to be blocked, and the roots starve, leading to plant death (Mountassif *et al*., 2008; Gaaied *et al*., 2019). 2,4-Dichlorophenoxyaceti acid is the active ingredient in more than 1 500 herbicide formulations globally (Islam *et al*., 2018) and is commonly applied to various crops, including wheat, barley, oats, maize, sugar cane, and grass (Gaaied *et al*., 2019). Since 2,4-D specifically targets dicots (plant seeds with two embryonic leaves, e.g., weeds), the herbicide does not affect monocots (plant seeds with one embryonic leaf, e.g., maize) (Song, 2014).

Although 2,4-D is considered non-persistent since it undergoes microbial degradation in soil (Islam *et al*., 2018), it was detected in the South African aquatic systems in recent years (Horn *et al*., 2019). Various endocrine disruptive effects have also been associated with 2,4-D exposure, such as spermatogenic disruption, seminiferous epithelial destruction, testicular dysfunction, and infertility have been reported in male mice (Harada *et al*., 2016). Furthermore, Mountassif *et al*. (2008) reported necrosis of seminiferous tubule cells in the testis of the desert rodent, *Jaculus orientalis*. Apart from the endocrine disruptive effects in animals, 2,4-D has also been found to increase the risk of infertility in humans due to dose-dependent inhibition of semen motility (Tan *et al*., 2016). Moreover, detectable levels of 2,4-D in the soil can affect non-target soil organisms of ecological importance, such as earthworms. This was evidenced by Hattab *et al*. (2015) who reported that 2,4-D exposure in *Eisenia andrei* not only affected the earthworm physiology but also induced oxidative stress by significantly altering the activity of antioxidant enzymes (SOD and CAT) and causing lipid peroxidation (LPO). Another author corroborated the findings that 2,4-D toxicity in non-target organisms may be related to the prevalence of oxidative stress (Ozcan Oruc *et al*., 2004). Exposure to 0.02, 0.051, 0.128, and 0.8 mg/L 2,4-D for 96 h led to the onset of oxidative damage in the form of LPO in zebrafish (*Danio rerio*) larvae (Gaaied *et al*., 2019)

2.2.2 Atrazine

Triazines elicit their herbicidal activity by blocking electron flow to the photosynthetic electron transport chain and inhibiting photosynthesis (Semren *et al*., 2018). Two commonly used triazine herbicides are atrazine and terbuthylazine. Atrazine (6-chloro-N-ethyl-NO-(1-methylethyl)-1,3,5-triazine-2,4-diamine) is a pre-emergent herbicide used for annual broadleaf and grass weeds (Griboff *et al*., 2014; Chen *et al*., 2015). Atrazine's triazine ring contributes to its degradation resistance (Blahová *et al*., 2013), making it highly persistent in the environment. Atrazine is slowly degraded in water, resulting in surface- and groundwater contamination by its leachates (Griboff *et al*., 2014). Many studies have reported on the endocrine disruptive effects of atrazine (Friedmann, 2002; Hayes *et al*., 2010; Wirbisky *et al*., 2016) and the herbicide is considered a known endocrine disruptor by the United States Environmental Protection Agency (Blahová *et al*., 2013). However, although the use of atrazine had been banned in the European Union (Sass & Colangelo, 2006), atrazine and herbicide formulations containing it as the active ingredient are still extensively applied to maize crops in South Africa—approximately 88% of the country's national atrazine use is for maize (Du Preez *et al*., 2005; Dabrowski, 2015a). Atrazine has been detected in water (< 5–1 570 ng/L) and soil (8.6–22.6 pg/g) in the South African environment (Rimayi *et al*., 2018; Degrendele *et al*., 2022). Nonetheless, atrazine is one of only two pesticides—endosulfan being the other—for which South Africa has water quality guidelines. The water quality guideline for atrazine in aquatic ecosystems is 10 µg/L as stipulated by the Department of Water Affairs and Forestry (DWAF, 1996). However, this guideline has not been updated since 1996, and there are also no guidelines for agricultural water used for irrigation or livestock watering.

2.2.3 Dicamba

Dicamba (3,6-dichloro-2-methoxybenzoic acid) is a broad-spectrum herbicide used for the control of annual and perennial broadleaf weeds in grain and cereal crops (Harp, 2010; Riter *et al*., 2021; Oseland *et al*., 2022). It is often also mixed with glyphosate and 2,4-D in large tanks and applied to post-emergent glyphosate-resistant weeds in soybean fields (Da Silva *et al*., 2020; Riter *et al*., 2021). The first commercial dicamba formulation was Banvel®, a dimethylamine dicamba salt, which was introduced in the 1960s (Riter *et al*., 2021). Just like 2,4-D, dicamba is a synthetic auxin-like herbicide and has the same mode of action—the manipulation of natural hormone responses in plants (Song, 2014; Riter *et al*., 2021). Even though new dicamba salt formulations have been developed, the herbicide is still highly volatile and prone to move towards off-target locations, which is especially influenced by wind speed and soil pH during application (Oseland *et al*., 2022). Dicamba is highly soluble in water and will readily leach or run-off into waterbodies (Harp, 2010) where it can cause biological effects on non-target aquatic species. For example, 48 hours of exposure to the commercial dicamba formulation Cowboy Elite SURCOS® induced oxidative stress in tadpoles of two amphibian species (*Scinax nasicus* and *Elachistocleis bicolor*). Dicamba was also previously found to cause steroid hormone receptor antagonism in the yeast anti-androgen screen assay (Westlund & Yargeau, 2017). Moreover, the herbicide can move to below the root zone, contaminating groundwater sources. This is especially the case if there has been sufficient rainfall or irrigation after application, or before dicamba can be degraded by soil microbiota (Ritter *et al*., 1996). No studies in literature could be found on the presence of dicamba in the South African environment.

2.2.4 Imidacloprid

Neonicotinoids are a newer class of insecticides with a chemical structure similar to nicotine (Kimura-Kuroda *et al*., 2012). Neonicotinoids are registered for use in more than 120 countries worldwide, emphasising their widespread use (Yan *et al*., 2020). They are considered a substitute for organophosphate (OP) pesticides (Kapoor *et al*., 2010) and are applied as seed treatments, foliar sprays, or through irrigation systems (Schaafsma *et al*., 2015). Neonicotinoids elicit their insecticidal mechanism of action by acting like false neurotransmitters that can bind to and activate the nicotine acetylcholine receptors of the central nervous system in insects (Topal *et al*., 2017; Tian *et al*., 2020). The binding results in the continuous stimulation of neurons, leading to invertebrate death (Želježić *et al*., 2016). There are currently seven different neonicotinoid varieties: imidacloprid, acetamiprid, nitenpyram, thiamethoxam, thiacloprid, clothianidin and dinotefuran. Imidacloprid (1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine), developed by Bayer Crop Science (Yan *et al*., 2020), was the first neonicotinoid insecticide that was introduced with commercial sales dating back three decades (Abu Zeid *et al*., 2019). Globally, it is the second most used agrochemical after glyphosate (Simon-Delso *et al*., 2015; Abu Zeid *et al*., 2019). Imidacloprid is used as a seed or soil treatment, foliar spray, tree trunk injection or stem brushing and drenching of flower bulbs (Njattuvetty Chandran *et al*., 2018; Abu Zeid *et al*., 2019; Contardo-Jara & Gessner, 2020). When applied as a seed coating, plant roots take up the active ingredient and during

germination and growth, it is translocated to all plant parts. As a result, the entire plant is toxic to insects (Contardo-Jara & Gessner, 2020). However, the use of imidacloprid as a seed coating poses an environmental risk. The insecticide has been detected in the Cape superfowl (*Pternistis capensis*), a bird species endemic to the Western Cape province of South Africa, following accidental ingestion of imidacloprid-treated barley seeds. Results from Tan and colleagues (2014) suggest that exposure to sublethal concentrations of imidacloprid alter the foraging abilities and predatory avoidance behaviours in honeybees. In addition, Tsvetkov *et al*. (2017) reported that in maize-growing regions of Canada exposure to neonicotinoids (including imidacloprid) under field-realistic conditions caused a reduction in honeybee health by increasing worker bee mortality and social immunity decline. Imidacloprid also induced oxidative stress in rock pigeons (*Columba livia domestica*), due to a reduction in SOD activity, leading to the accumulation of superoxide and peroxide radicals, and subsequent peroxidation of cell membrane lipids (Abu Zeid *et al*., 2019). Although the European Union has prohibited the use of imidacloprid as a seed treatment because of its effects on non-target organisms the use of imidacloprid as a seed coating in South Africa has not yet been reassessed (Botha *et al*., 2018).

2.3 Selected biological effects associated with agrochemical exposure

If bioavailable, agrochemical mixtures can elicit several biological effects. The most relevant endpoints for non-target organism, including humans and wildlife, are endocrine disruption (Kass *et al*., 2020; Martyniuk *et al*., 2020), oxidative stress and damage (Jabłońska-Trypuć *et al*., 2017; Sule *et al*., 2022), and the inhibition of non-neuronal AChE (Toledo-Ibarra *et al*., 2021).

2.3.1 Endocrine disruption

2.3.1.1 The endocrine system

The endocrine system is comprised of three main components: i) endocrine glands distributed throughout the body (Garcia-Reyero, 2018); ii) signalling molecules, called hormones, which are secreted into the circulatory system by the endocrine glands and transported to their target organ via the bloodstream to elicit specific responses (Kaltenbach, 1988; Gore *et al*., 2015); and iii) hormone receptors located in various tissues and organs in the body that respond to hormone signals (La Merrill *et al*., 2020; US EPA, 2022). The endocrine glands include the hypothalamus, pituitary, pineal, thyroid, and parathyroid, thymus, pancreas, adrenal, gonadal glands (located in the testes and ovaries of males and females, respectively), and the placenta (during pregnancy) (Gore *et al*., 2015; Heindel & Blumberg, 2019) (**Figure 1**). Within the endocrine system there is a level of hierarchy: hormonal regulation in endocrine glands is controlled by the brain. In the neurosecretory cells of the hypothalamus signals are integrated and processed, releasing hormones as a result. After these hormones reach the pituitary gland, further hormonal synthesis takes place and the release of hormones in the thyroid, adrenal glands, or gonads is induced (Kleine & Rossmanith, 2016). The endocrine system is also interconnected with other parts of the body, with the gastrointestinal

tract (GIT) representing the largest endocrine organ in the body by releasing peptide hormones and regulating metabolic homeostasis (Huang *et al*., 2020b). The nervous and immune systems also include hormonal feedback mechanisms since hormones act on nerve, immune and endocrine cells. Nerves reach nerve, immune and endocrine cells; and the immune system has receptors at nerve and endocrine cells (Kleine & Rossmanith, 2016). Moreover, "clandestine organs" of the endocrine system have also been described due to their interconnectedness and feedback mechanisms and include the gut microbiome and stress system (Garcia-Reyero, 2018).

All the components of the endocrine system work together to regulate essential biological functions within the body, such as reproduction, metabolism, water balance, feeding, and growth (Karthikeyan *et al*., 2019; Farounbi & Ngqwala, 2020). Of all the different hormones, steroid hormones are of particular interest. Three important classes of steroid hormones include oestrogens, androgens, and glucocorticoids which are responsible for essential physiological processes such as reproduction, stress management, salt and glucose balance (Sonneveld *et al*., 2006). These endogenous hormones circulate in the bloodstream and evoke their responses at very low, but physiologically relevant, concentrations (usually in the parts per billion or trillion range) (La Marrilla *et al*., 2020). This is due to the remarkable affinity with which the hormones bind to their receptors (Gore *et al*., 2015). Steroidal hormones have a relatively simple chemical structure and are lipophilic in nature. Consequently, their regulatory pathways are easily influenced by especially lipophilic environmental chemicals (Sonneveld *et al*., 2006). This is worrisome as elevated or depressed hormone levels can result in reproductive dysfunction (Gore *et al*., 2015), for example intersex in fish species (Barnhoorn *et al*., 2004), impaired spermatogenesis in wild antelope (Bornman *et al*., 2010), and an increase in number of infertile and banded eggs in crocodiles (Arukwe *et al*., 2016).

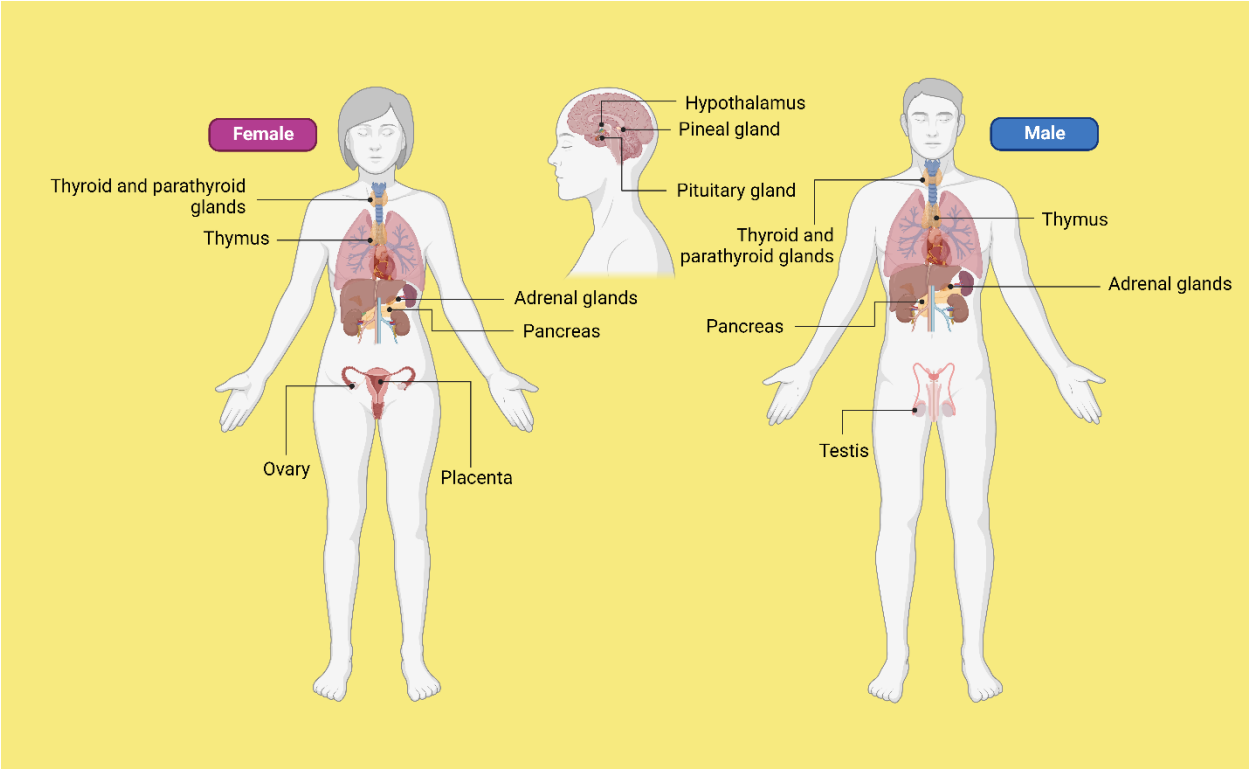

Figure 1 The endocrine system in females and males (Hiller-Sturmhöfel & Bartke, 1998) (created in BioRender.com).

2.3.1.2 Endocrine disrupting chemicals

Chemicals or mixtures of chemicals that can interfere with the normal functioning of the endocrine system are called endocrine disrupting chemicals (EDCs) (Gore *et al*., 2015). Recently, La Merrill and colleagues (2020) published an Expert Consensus Statement in *Nature Reviews Endocrinology* in which ten key characteristics of EDCs, based on their mechanisms of action, were described. These key characteristics include (1) binding to and/or activating hormone receptors in the place of endogenous hormones (agonism); (2) inhibiting or blocking the binding of endogenous hormones to hormone receptors (antagonism); (3) alterations in hormone receptor expression; (4) alterations of signal transduction in hormone-responsive cells [e.g., changes in protein or ribonucleic acid (RNA) expression]; (5) epigenetic modifications in hormone-producing or -responsive cells [e.g., deoxyribonucleic acid (DNA) methylation]; (6) changes in hormone synthesis; (7) alterations in the transportation of hormones across cell membranes; (8) changes in hormone distribution or circulating levels; (9) alternations in hormone breakdown/clearance; and (10) changes in the fate or hormone-producing or -responsive cells. Endocrine disrupting chemicals are biologically active at very low concentrations—comparable to those of endogenous hormones (Archer & Van Wyk, 2015; Heindel & Blumberg, 2019). Since these endogenous cellular signalling pathways of hormones are already active, they are targets for EDCs (Heindel & Blumberg, 2019). Endocrine disrupting chemicals can either be of natural origin (e.g., 17β-oestradiol (E_2), oestrone, phytoestrogens) (Pojana *et al*., 2007; Sim *et al*., 2022) or synthetic (e.g., plasticisers, pharmaceuticals and personal care products, flame retardants, and pesticides) (Aït-Aïssa *et al*., 2010; Archer *et al*., 2017; Gore *et al*., 2015; Farounbi &

Ngqwala, 2020). Several pesticides have been reported to cause endocrine disruption in non-target biota as reviewed by Horak *et al*. (2021).

2.3.2 Oxidative stress

2.3.2.1 Reactive oxygen species, antioxidant defence system, and lipid peroxidation

Reactive oxygen species (ROS) originate from the partial reduction of oxygen and are endogenously produced as by-products of aerobic metabolic processes, including mitochondrial respiration (Ray *et al*., 2012; He *et al*., 2017). Reactive oxygen species include both free radical [superoxide ($O_2^{\bullet-}$) and hydroxyl radicals ($^{\bullet}OH$)] and non-free radical molecules [hydrogen peroxide (H_2O_2)] (Phaniendra *et al*., 2015; Pisoschi & Pop, 2015). Under normal physiological conditions, ROS play an essential role in cellular homeostasis, maintaining biological functions, such as the regulation of gene expression, cell proliferation, survival, apoptosis, and tissue development (Jabłońska-Trypuć *et al*., 2017; Pisoschi & Pop, 2015; Ventura *et al*., 2015). However, exposure to xenobiotics can increase the production of ROS: when xenobiotics undergo phase I and II biotransformation reactions mediated by cytochrome P450 (CYP450) monooxygenases and other detoxification enzymes in the liver, ROS are also produced as by-products (Ray *et al*., 2012; He *et al*., 2017). Excessive ROS production induces oxidative stress—a physiological imbalance between the production and accumulation of ROS and a biological system's capability to neutralise and eliminate these ROS via the antioxidant defence system (Archibong *et al*., 2018; Burella *et al*., 2018; Jabłońska-Trypuć *et al*., 2017). The antioxidant defence system is comprised of enzymatic and non-enzymatic components that work together to neutralise ROS, preventing oxidative stress and damage (Diken *et al*., 2017 Jabłońska-Trypuć *et al*., 2017; Nwani *et al*., 2013; Sparks *et al*., 2019) (**Figure 2**). The reduced form of glutathione (GSH) is the main antioxidant in the body (Ansari & Ansari, 2014) and maintains cellular redox homeostasis by either (i) directly neutralising ROS via electron donation or (ii) acting as the substrate for several antioxidant enzymes (Serdar, 2019).

In terms of enzymatic components, SOD and CAT form the first line of defence against ROS (Nwani *et al*., 2013). During detoxification of ROS, SOD converts superoxide radicals into H_2O_2 and molecular oxygen (O_2) (McCord & Fridovich, 1969). Catalase converts the resulting H_2O_2 into water (H_2O) and O_2 (Chance, 1948) (**Figure 2**). Overall, the neutralisation of ROS and detoxification of pesticides initially increases SOD and CAT activity. However, over time reduced enzyme activity could indicate that the antioxidative responses are overwhelmed and can no longer mitigate the damage caused by ROS generation, leading to the onset of oxidative stress (Khalil, 2015; Mossa *et al*., 2015). When ROS are unable to successfully scavenge electrons they interact with important endogenous cellular targets, including proteins, nucleic acids, lipids, and sugars (Agrawal & Sharma, 2010; Archibong *et al*., 2018) (**Figure 2**). This causes cell injury to occur at a biomolecular level and is known as oxidative damage (Halliwell & Whiteman, 2004). Oxidative damage could eventually lead to cell death (Ansari & Ansari, 2014).

One example of oxidative damage is LPO: the oxidative degradation of lipid components, such as polyunsaturated fatty acids, within the cellular membranes of an organism (Bindoli, 1988). Lipid peroxidation is initiated when ROS bind to the double-bonded carbons of polyunsaturated fatty acids, leading to the formation of lipid hydroperoxides (Cortés-Iza & Rodríguez, 2018). These lipid hydroperoxides decompose the double-bonded carbons in cellular membranes (Dar *et al*., 2015). Consequently, structural cell membrane integrity and fluidity are reduced by an increase in permeability and instability (Baruah & Chaurasia, 2020; El-Gendy *et al*., 2010).

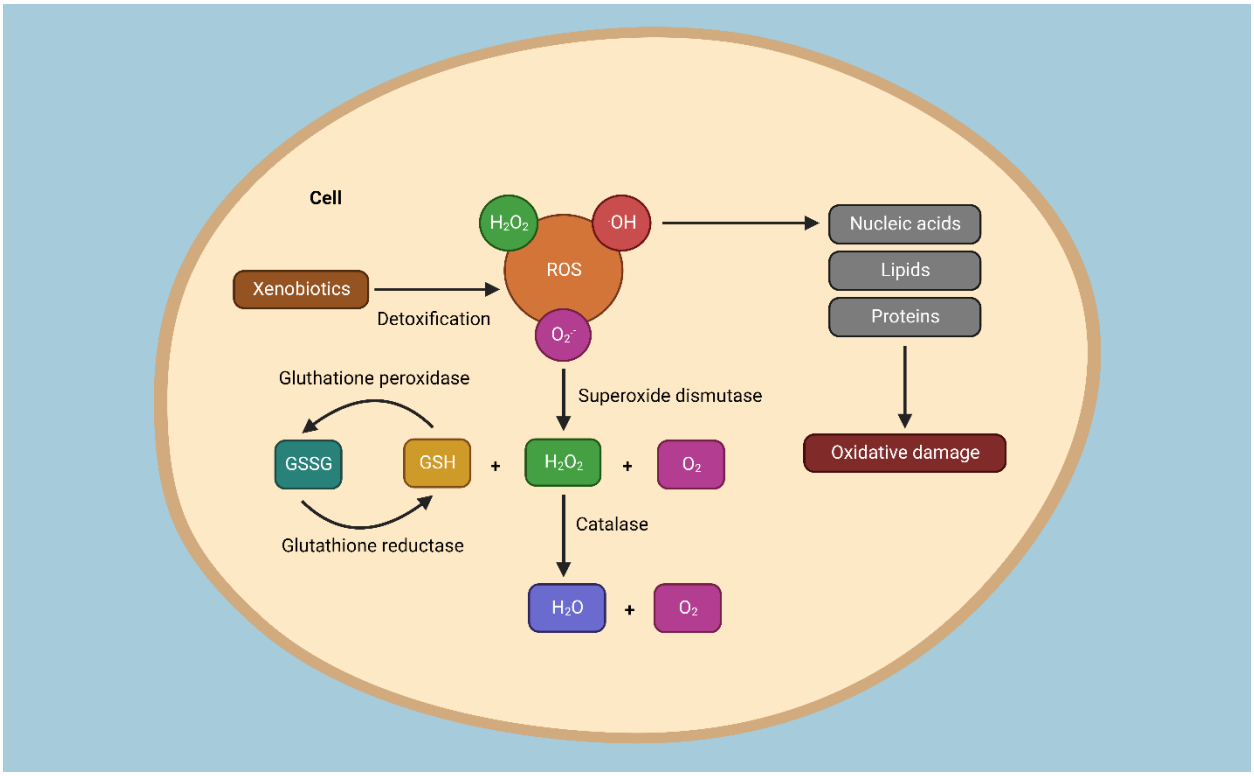

Figure 2 Reactions of the antioxidant defence system inside a cell. GSH: reduced form of glutathione; GSSG: oxidised form of glutathione (glutathione disulphide); H_2O: water; H_2O_2: hydrogen peroxide; O_2: molecular oxygen; $O_2^{\bullet-}$: superoxide radical; $^{\cdot}OH$: hydroxyl radical; ROS: reactive oxygen species (Benzie, 2000; Ray *et al*., 2012; He *et al*., 2017; Pisoschi *et al*., 2021) (created in BioRender.com).

2.3.3 Acetylcholinesterase activity

2.3.3.1 The non-neuronal cholinergic system (NNCS)

The cholinergic system collectively refers to all cells, enzymes, transporters, and receptors involved in the synthesis, transmission, and degradation of acetylcholine (ACh). These components can be present inside the nervous system (i.e., neuronal cholinergic system) or outside of the nervous system (i.e., non-neuronal cholinergic system; NNCS) (Wessler *et al*., 1998). Although there are differences between the neuronal cholinergic system and the NNCS, their signalling pathways are comparable. Acetylcholine is synthesised in almost all living cells (Wessler & Kirkpatrick, 2008) from choline and acetyl co-enzyme A by the enzyme

choline acetyltransferase (Gordan *et al*., 2015). Signal transmission is modulated by ACh via two acetylcholine receptors (AChR) which are also expressed in non-neuronal cells: the nicotinic and muscarinic receptors (Wessler & Kirkpatrick, 2008; Gordan *et al*., 2015; Gu & Wang, 2021). These receptors interact with almost all cellular signalling pathways (Wessler *et al*., 2001). Yet, for many organs the expression and functioning of the NNCS are not clear yet, but it is known to have important functions in the integumentary (especially skin cells), respiratory, cardiovascular, immune, musculoskeletal, urogenital, reproductive, and digestive systems (Wessler *et al*., 1998; Beckmann & Lips, 2013). Some of these functions include gene expression, cell proliferation, differentiation, cytoskeletal organisation, cell-cell contact, ciliary activity, secretion, and absorption (Wessler *et al*., 2001).

In neuronal cells, after ACh has activated the AChR, eliciting a response, it disconnects from the receptor and is hydrolysed to choline and acetate by the cholinergic enzyme AChE (Quinn, 1987; Lionetto *et al*., 2013). Termination of ACh is essential as it ensures the signal is only sent once, with newly synthesised ACh transmitting the next signal. If old ACh molecules are not hydrolysed, they will accumulate and continuously stimulate signal transmission (Donkin & Williams, 2000). However, although the release of neuronal AChE is well-understood, this is not the case for non-neuronal AChE for which the mechanisms of release are not clear yet (Wessler & Kirkpatrick, 2008). Nonetheless, variable amounts of AChE are released and found in several organs outside of the nervous system: heart, kidney, ovary cells, vagina, placenta, pancreas, intestines, and liver (Berninsone *et al*., 1989; Wessler *et al*., 1998; García-Ayllón *et al*., 2012; Beckmann & Lips, 2013; Pérez-Aguilar *et al*., 2015). In these non-neuronal cells, AChE is also responsible for breaking down ACh into choline and acetate (Wessler *et al*., 2001).

Organophosphate pesticides are known to elicit their activity by binding to neuronal AChE, reducing its activity and causing ACh accumulation (Sturm *et al*., 2007; Sandoval-Herrera *et al*., 2019). Acetylcholinesterase inhibition has also been reported for certain pyrethroid and neonicotinoid insecticides (Tu *et al*., 2012; Topal *et al*., 2017; Qi *et al*., 2018), and glyphosate-based herbicides (Bali *et al*., 2019; Pala, 2019). Pesticides may therefore also interfere with the activity of non-neuronal AChE. Since the NNCS plays a role in cell growth, adhesion, migration, differentiation, absorption, and secretion (Wessler *et al*., 1999; Beckmann & Lips, 2013) any impairment or dysregulation (including AChE inhibition) can have pathogenic effects causing several diseases as reviewed by Beckmann and Lips (2013). Acetylcholinesterase inhibition in the liver of fish has been widely used as a biomarker of pesticide toxicity (Capkin *et al*., 2014; Jindal & Kaur, 2014; Sharbidre *et al*., 2011). Inhibition of AChE in liver and intestinal cells, therefore, indicates damage to non-neuronal cholinergic transmission in important detoxification organs.

2.4 The use of cell-based methods to investigate biological effects

2.4.1 Chemical mixtures

As previously mentioned, pesticides occur in combination with other agrochemicals. Consequently, known and unknown chemicals end up in environmental matrices (i.e., soil, water and sediment) at concentrations that are variable in time and space (Archer & Van Wyk, 2015; Legradi *et al*., 2018). These chemicals can also have similar or dissimilar mechanisms of action (Legradi *et al*., 2018). Moreover, not only agrochemicals end up in the environment. There are more than 67 million known organic and inorganic compounds (Connon *et al*., 2012) that encompass many classes, such as pharmaceuticals, personal care products, industrial chemicals, surfactants, synthetic hormones, and agrochemicals, including pesticides. Single compounds can react differently when combined with other compounds (Archer & Van Wyk, 2015). Consequently, exposure to a mixture of chemicals is the most common and realistic exposure scenario in the environment (Connon *et al*., 2012). In terms of mixture effects, it is generally assumed that chemicals with the same mode of action follow the concept of concentration addition, while chemicals with different modes of action produce effects independently (independent action) (Escher *et al*., 2021). Although analytical approaches are used for the chemical characterisation of environmental samples, it is impossible to detect, analyse and quantify every chemical present in the environment (Connon *et al*., 2012). Moreover, the phenomena of matrix suppression can also interfere with the analysis (Escher *et al*., 2020). The presence of a chemical does not provide information regarding the bioavailability and potential biological hazard to organisms. In many cases, environmental concentrations of chemicals fall well below the limit of detection of the analytical instrument (Neale *et al*., 2020). It is therefore important to also investigate the biological effects of these chemical mixtures to ensure human and environmental health (Taxvig *et al*., 2013).

2.4.2 Effects-based methods

Effects-based methods (EBMs) are a useful tool to overcome the limitations of instrumental analysis and detect biological effects, such as xenobiotic metabolism, endocrine disruption, oxidative stress, and AChE inhibition, induced by complex environmental mixtures (Neale *et al*., 2015; Legradi *et al*., 2018). Effects-based methods are defined as bioanalytical methods that are used for the detection and quantification of effects elicited by chemical mixtures (Brack *et al*., 2019). Based on this definition, EBMs can be divided into three main groups: (i) bioassays (*in vitro* and *in vivo* tests), (ii) biomarkers (molecular, biochemical, cellular, and physiological indicators), and (iii) ecological methods (functional species traits) (Wernersson *et al*., 2015; Brack *et al*., 2019). Effect-based methods are also described as mode-of-action-specific tests since they measure specific toxicological endpoints and target different levels of biological organisation—from molecular to cellular, tissue to organ, and population to community level (Connon *et al*., 2012; Brack *et al*., 2019). *In vitro* bioassays are single-cell systems that measure effects at the level of cellular response pathways, such as receptor-mediated effects, mutagenicity, genotoxicity (DNA damage), inhibition of photosynthesis, induction of xenobiotic metabolism, activation of adaptive stress response pathway (e.g., oxidative stress), and cytotoxicity (Sonneveld *et al*., 2006; Connon *et al*., 2012; Escher *et al*., 2014; Neale

et al., 2015; Brack *et al*., 2019). *In vivo* bioassays measure organism-level effects, such as growth, fecundity, and mortality in whole living species (e.g., green algae, water flea, and larval fish) (Connon *et al*., 2012; Wernersson *et al*., 2015). However, although effects at the cellular level do not necessarily translate into higher level effects, they are key steps in adverse outcome pathways (Neale *et al*., 2015). Moreover, it is important to note that the use of EBMs to screen for biological effects is a Tier 1 approach focused on specific pathways (Costa *et al*., 2011). Therefore, when using EBMs, a battery of bioassays should be used that evaluate a range of different end-points—the so-called weight of evidence approach (Connon *et al*., 2012). If a bioassay reveals an effect, it can be further investigated using fractionation procedures and analytical methods to potentially identify chemicals (Brack, 2003; Legradi *et al*., 2018). Although EBMs include both *in vitro* and *in vivo* bioassays (Connon *et al*., 2012), only *in vitro* bioassays were employed in this study as *in vivo* tests were beyond the scope of the study.

One of the challenges of investigating EDCs and other environmental mixtures is that they are biologically active at very low concentrations (Archer & Van Wyk, 2015). This limitation can be overcome with *in vitro* cell lines. *In vitro* bioassays are highly sensitive and able to detect responses elicited by low concentrations of compounds in environmental mixtures (Horn *et al*., 2020). The use of tissue cultures also has several other advantages: (i) they are non-animal models; (ii) cell lines grow easily and divide rapidly; (iii) the physicochemical environment of cells can be easily manipulated; (iv) cells can be directly exposed to the chemical; (v) the concentration of a chemical delivered to the entire cell population can be controlled in terms of the amount delivered; (vi) responses can be measured in the absence of chemical metabolites and *in vivo* homeostatic mechanisms; (vii) they have the ability to provide information on basic mechanistic processes; (viii) *in vitro* bioassays are good predictors of certain *in vivo* effects (e.g. oestrogenic and androgenic activity); (ix) they allow for the high-throughput screening of environmental samples in a laboratory to identify toxic hazards and pollution "hotspots"; (x) the effect of a wide range of environmental matrices (e.g., surface water, effluent, soil, sediment) can be assessed; and (xi) tissue cultures are available from various mammalian models, including humans (Harry *et al*., 1998; Sonneveld *et al*., 2006; Costa *et al*., 2011; Wernersson *et al*., 2015).

There are several different *in vitro* effects-based methods to detect toxicological responses associated with environmental mixtures, including reporter gene bioassays that evaluate the induction of xenobiotic metabolism [e.g. H4IIE-*luc* bioassay for AhR activity] and endocrine disruption [e.g. MDA-kb2 bioassay for (anti-)androgenic activity and T47D-KB*luc* bioassay for (anti-)oestrogenic activity]; and enzymatic bioassays to detect oxidative stress responses [e.g., ROS production, SOD content, and CAT activity] and effects on the NNCS (i.e., AChE activity).

2.4.2.1 Bioassays indicative of receptor-mediated effects

One type of *in vitro* EBM is cell-based reporter gene bioassays which detect effects mediated through receptors that belong to the nuclear receptor superfamily of transcription factors, including non-steroidal nuclear receptors (e.g., AhR) and steroidal nuclear receptors (e.g., oestrogen, androgen, glucocorticoid, progesterone, and mineralocorticoid receptors) (Sonneveld *et al*., 2006; Connon *et al*., 2012; Timmermans *et al.*, 2019). Under normal physiological conditions, steroidal hormones such as oestrogens, androgens, and glucocorticoids act as nuclear hormone receptor ligands that bind to their respective receptors upon entry into the cells. The receptors are activated and form a hormone-receptor complex that enters the cell nucleus, binding to specific recognition sequences, called hormone response elements, located in the promoter region of target genes. In the presence of receptor-interacting proteins (cofactors), the DNA-bound receptor activates transcription of the target gene, and messenger RNA is translated into a protein (Legler *et al*., 1999; Sonneveld *et al*., 2006) (**Figure 3**).

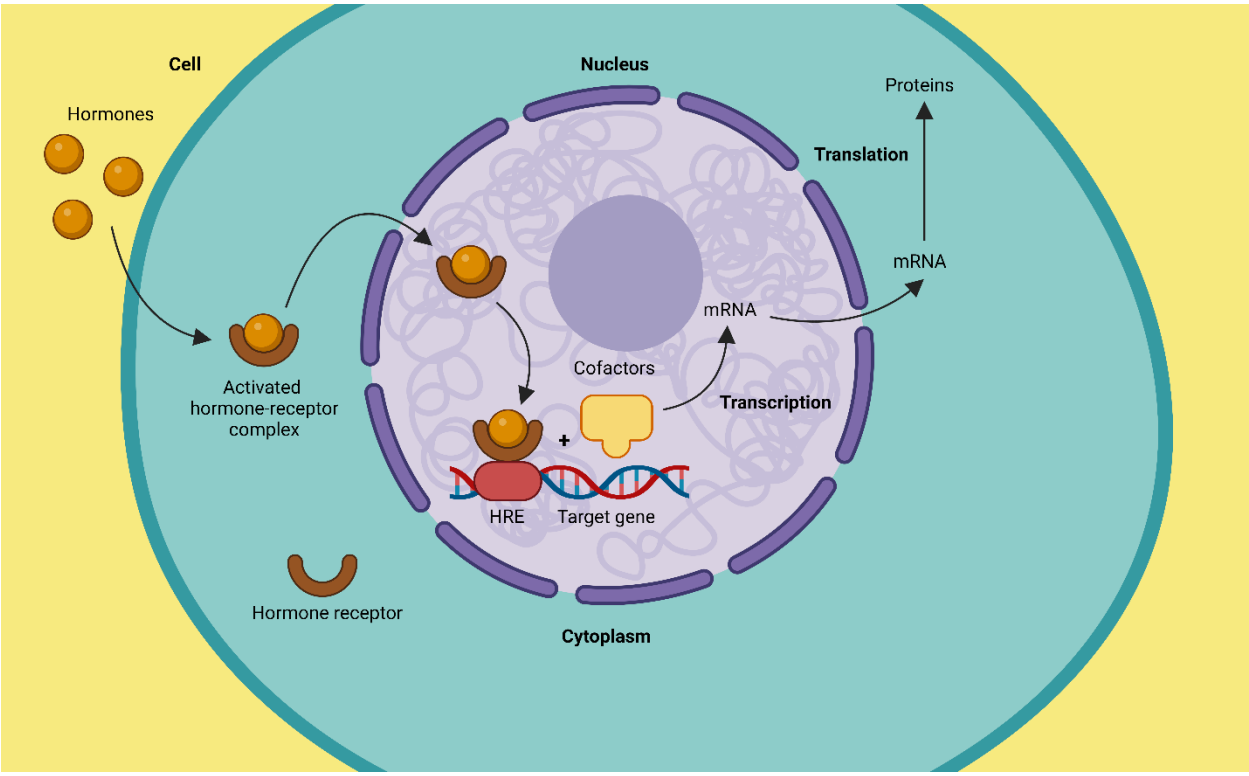

Figure 3 A generalised genomic pathway of steroidal nuclear hormone receptors that lead to the synthesis of proteins. Upon entry into the cell, hormones bind to the hormone receptor forming an activated hormone-receptor complex. This complex translocates into the cell nucleus where it binds to the hormone response element (HRE). In the presence of cofactors, transcription of target genes is activated and translation of messenger RNA (mRNA) into proteins occur (Legler *et al*., 1999; Sonneveld *et al*., 2006) (created in BioRender.com).

This mechanism of action was exploited and used to develop reporter gene bioassays which are based on this principle with a few modifications: cells had been genetically modified in such a way that the hormone response elements are linked to the gene of an easily measurable protein, the so-called reporter gene

(Jausons-Loffreda *et al*., 1994) (**Figure 4**). The most common reporter gene used is the *luc* gene isolated from a firefly (*Photinus pyralis*) and which expresses luciferase (a luminescent enzyme) (Sonneveld *et al*., 2006). In a reporter gene bioassay, a ligand binds to the nuclear receptors leading to the expression of the reporter gene (*luc*) and synthesis of the enzyme luciferase. In the presence of the substrate luciferin, light is emitted which can be measured spectrophotometrically (**Figure 4**). The amount of light emitted and quantified, is directly proportional to the number of ligands present (Denison *et al*., 2004). Depending on the receptor under investigation, the resulting response [xenobiotic, (anti-)oestrogenic, (anti-)androgenic and (anti-)glucocorticoid activity] is expressed in terms of a corresponding reference compound as bioassay equivalents (Könemann *et al*., 2018). In this study, three types of reporter gene bioassays assessing different endpoints were used: (i) H4IIE-*luc* bioassay indicative of xenobiotic metabolism through the AhR; (ii) MDA-kb2 bioassay for AR and GR activity; and (iii) T47D-KB*luc* bioassay for oestrogen receptor (ER) activity.

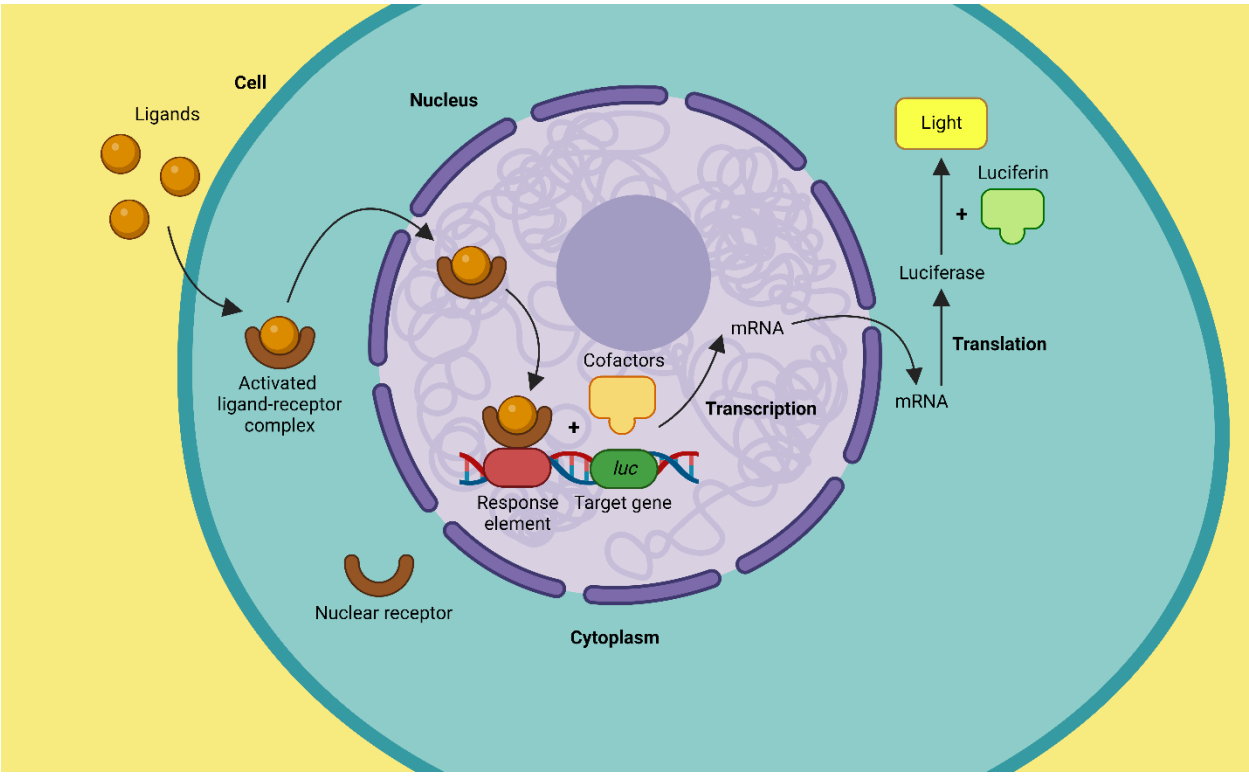

Figure 4 Principle of the reporter gene bioassay in which cells have been genetically modified to express the *luc* gene. A ligand binds to the nuclear receptor forming an activated ligand-receptor complex which translocates into the cell nucleus. The complex binds to the response element located downstream of the *luc* gene, activating transcription. The enzyme luciferase is produced and when the substrate luciferin is added, light is emitted. The amount of luminescence can be quantified spectrophotometrically (Legler *et al*., 1999; Denison *et al*., 2004; Sonneveld *et al*., 2006) (created in BioRender.com).

2.4.2.1.1 Xenobiotic metabolism and the aryl hydrocarbon receptor (AhR)

Xenobiotic metabolism describes the biochemical process during which xenobiotics are transformed inside the cell to allow detoxification of the compounds (Newman, 2015). Phase I and II biotransformation describes the biphasic nature of xenobiotic metabolism that takes place in the liver (Omiecinski *et al*., 2011). Phase I reactions involve hydrolysis, reduction, and oxidation of xenobiotics and CYP450 are the main enzymes responsible for these biotransformations (Newman, 2015). Many phase I products are more water soluble than the parent compound and as a result, are easily excreted. However, if compounds are not excreted during phase I, they are readily available for further reactions and undergo conjugation during phase II (Omiecinski *et al*., 2011). Xenobiotic metabolism is a useful measure of the toxicity of chemicals since these effects occur at much lower concentrations than those causing cell damage or death (Neale *et al*., 2015). One of the most well-known cell-based systems used to detect xenobiotic metabolism is the H4IIE-*luc* cell line which was developed by Aarts *et al*. (1993) using recombinant rat hepatoma H-4-II-E cells stably transfected with a dioxin-responsive *luc* reporter gene and showing AhR-controlled luciferase expression.

The AhR is a ligand-dependent transcription factor responsible for xenobiotic metabolism. However, the AhR also regulates genes involved in cellular proliferation and differentiation in the liver and ovary development, immune system homeostasis, and tumour development; plays a role in atherogenesis, and regulates antibody secretion (Barouki *et al*., 2007; Takeuchi *et al*., 2008; Ghisari *et al*., 2015; Tian *et al*., 2015). Aryl hydrocarbon receptor ligands stimulate the transcription of two genes, including phase I and II xenobiotic metabolism enzymes, which are part of the CYP450 family: *cyp1a1* and *cyp1b1* (Hankinson, 1995; Ghisari *et al*., 2015). The AhR is most well-known for mediating the toxicological effects of hydrophobic dioxin-like compounds, such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (Ohtake *et al*., 2009; Omiecinski *et al*., 2011), dioxin-like polychlorinated biphenyls (PCBs) and some polyaromatic hydrocarbons (PAHs) (Connon *et al*., 2012). Since some environmental chemical contaminants are AhR ligands (Leskinen *et al*., 2008), the H4IIE-*luc* cells can be used to assess both AhR agonistic and antagonistic activity of environmental mixtures (Aarts *et al*., 1995; Ghisari *et al*., 2015; Doan *et al*., 2019). The mechanism of action of the AhR is similar to that of steroidal nuclear receptors (**Figure 3**), however, there are a few differences. When a ligand (e.g., xenobiotic) binds to the AhR in the cytoplasm, the AhR-ligand complex moves into the cell nucleus and heterodimerises with the AhR nuclear translocator (ARNT) protein to form the ligand AhR-ARNT complex (Ohtake *et al*., 2003; Baba *et al*., 2005; Ghisari *et al*., 2015). This complex binds to the xenobiotic response element (XRE) which is in the upstream promotor region of the AhR responsive gene *cyp1a1*, increasing gene expression (Aarts *et al*., 1995). Once transcriptional regulation is complete, the AhR is transported to the cytosol for degradation by proteasomes (Barouki *et al*., 2007) (**Figure 5**).

Apart from its role in xenobiotic metabolism, the AhR also plays an essential role in female reproduction and interference as suggested by Baba *et al*. (2005). The AhR plays a key role in the regulation of an important enzyme responsible for ovarian oestrogen synthesis in the oestrus cycle: ovarian CYP450 aromatase (Baba *et al*., 2005). There is also crosstalk between the signalling pathways of the AhR and the ER, since an agonist-activated AhR-ARNT dimer directly associates with the α- and β-ER, resulting in the activation of unliganded ER and co-activator p300 to oestrogen-responsive gene promoters. This activates transcription and oestrogenic effects (Ohtake *et al*., 2003). Aryl hydrocarbon receptor ligands could indirectly modulate the function of hormone receptors and cause endocrine disruptive effects. As a result, it is important to also consider AhR activity when investigating endocrine disruptive effects (Ghisari *et al*., 2015).

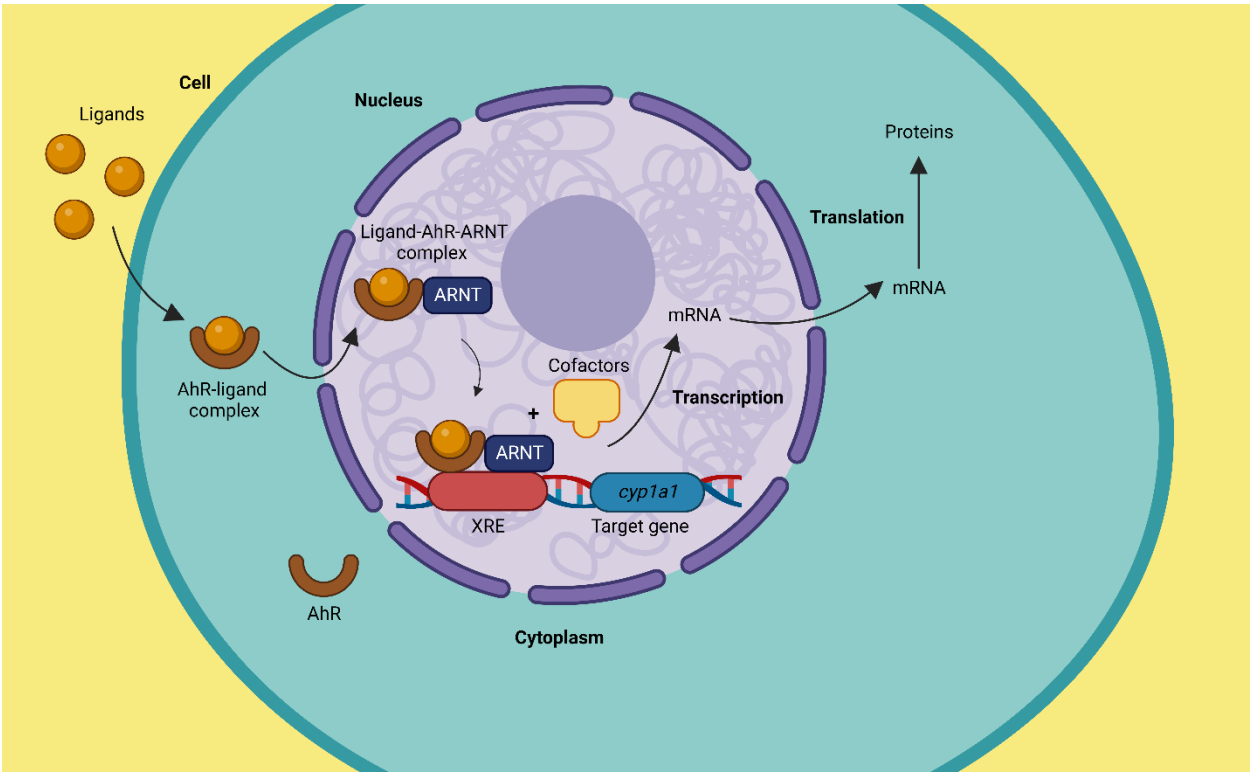

Figure 5 Genomic pathway of the aryl hydrocarbon receptor (AhR). After receptor binding of ligands (e.g., xenobiotics) to the AhR, the ligand-receptor complex moves into the cell nucleus where it heterodimerises with the AhR nuclear translocator (ARNT) protein to form the ligand AhR-ARNT complex. The complex binds to the xenobiotic response element (XRE) which is in the upstream promotor region of the AhR responsive gene *cyp1a1*. The target gene is transcribed into messenger RNA which is translated into proteins (e.g., phase I and II biotransformation enzymes) (Aarts *et al*., 1995; Ohtake *et al*., 2003; Baba *et al*., 2005; Ghisari *et al*., 2015; Larigot *et al*., 2018) (created in BioRender.com).

2.4.2.1.2 Androgens and the androgen receptor (AR)

Androgens are natural male sex steroid hormones synthesised from cholesterol and pregnenolone in the steroidogenesis pathway. Androgen production is regulated by the hypothalamic-pituitary-gonadal axis

with testosterone being the main circulating androgen in men (Debes & Tindall, 2002). The primary function of androgens is regulation of the male reproductive system, including sexual differentiation of the male reproductive tract and accessory reproductive organs, development and functioning of the prostate, male pubertal maturation, and maintenance of secondary sex characteristics in adults: regulation of metabolism, muscle growth, bone formation, endocrine function, and the maintenance of musculoskeletal, cardiovascular, and immune systems (Wilson *et al*., 2002; Davey & Grossmann, 2016; Feng & He, 2019; Huanyu *et al*., 2022). Androgen function is regulated by binding to the ligand-dependent AR of which the gene is located on the X chromosome (Davey & Grossmann, 2016). The AR is a member of the steroid hormone nuclear receptor family (Davey & Grossmann, 2016) and is mainly expressed in the prostate and adrenal glands (Huanyu *et al*., 2022). Testosterone is the main endogenous androgen in the body that binds to the AR (Feng & He, 2019) with the majority of testosterone produced by Leydig cells in the testes (Debes & Tindall, 2002). Only small amounts of testosterone are produced by the adrenal glands (Feng & He, 2019; Debes & Tindall, 2002). Another endogenous androgen is 5α-dihydrotestosterone (DHT) which is synthesised from testosterone through the enzymatic activity of 5α-reductase found in the skin, the prostate and scalp (Feng & He, 2019). Apart from natural androgens to which the AR responds, there are several sources of exogenous androgens that can also bind to the AR (Connon *et al*., 2012) such as synthetic androgens which are used as growth regulators in livestock. This is worrisome as exposure to environmental (anti-)androgens have shown to affect androgen-dependent play behaviour in males Sprague-Dawley rats (Hotchkiss *et al*., 2002). Moreover, short-term exposure to methyltestosterone and decreases steroid levels in a concentration-dependent manner but increased vitellogenin levels in male adult zebrafish (*Danio rerio*) (Andersen *et al*., 2006). Androgens acting through the AR have also been implicated in prostate cancer (Debes & Tindall, 2002).

2.4.2.1.3 Glucocorticoids and the glucocorticoid receptor (GR)

Glucocorticoids are a class of steroid hormones that are also produced and released by the adrenal cortex under the control of the hypothalamic-pituitary-adrenal axis (Rhen & Cidlowski, 2005). Glucocorticoids are essential for life and their activity is regulated by the GR which is ubiquitously expressed by almost all cells in the body (Revollo & Cidlowski, 2009). Between 1 000 and 2 000 genes in the body are subject to GR-mediated regulation (Timmermans *et al*., 2019). After synthesis, glucocorticoids are secreted into the bloodstream, binding to plasma proteins. Cortisol is the most important endogenous glucocorticoid and when biologically active cortisol binds to the glucocorticoid receptor (Rhen & Cidlowski, 2005; Timmermans *et al*., 2019). The hormone-receptor complex translocates into the nucleus, heterodimerises, and elicits changes in gene expression by binding to the glucocorticoid response element (Rhen & Cidlowski, 2005). Apart from directly interacting with DNA, changes in gene expression can also occur through protein-protein interactions with other transcription factors. Glucocorticoids are part of the body's adaptive response that allows us to cope with environmental and physiological stress (Revollo & Cidlowski, 2009). The main role of endogenous glucocorticoids is immunomodulation (Timmermans *et al*., 2019) by

upregulating the expression of anti-inflammatory proteins and down-regulating the expression of pro-inflammatory proteins (Garcia-Reyero, 2018). However, glucocorticoids are also involved in several other physiological processes, including glucose metabolism, mood and cognitive functions, bone mineralisation, and reproduction (Revollo & Cidlowski, 2009; Timmermans *et al*., 2019). Due to their great immunosuppressive power, synthetic glucocorticoids (e.g., prednisolone, fluticasone, and dexamethasone) have also been developed and are mainly used for the treatment of several diseases (Timmermans *et al*., 2019), including asthma, rheumatoid arthritis, eczema, and even psychological brain disorders (Revollo & Cidlowski, 2009). Synthetic glucocorticoids are more potent than endogenous glucocorticoids and often activate the GR better due to having higher affinity. Synthetic glucocorticoids that originate from the environment could affect the binding of endogenous glucocorticoids to the GR, leading to severe complications.

Androgen- and glucocorticoid-mediated effects of environmental mixtures can be assessed using the stable human breast carcinoma cell line MDA-kb2. This cell line was developed from MDA-MB-453 cells almost two decades ago by Wilson *et al*. (2002) and is a useful tool for studying both AR and GR activity (Aït-Aïssa *et al*., 2010; Klopčič *et al*., 2015). The MDA-kb2 cell line was transformed with an androgen-responsive luciferase reporter plasmid driven by the mouse mammary tumour virus (MMTV) promoter. Since the MDA-kb2 cell line expresses both the AR and GR, compounds that act through either of these two receptors will activate the MMTV luciferase reporter gene (Wilson *et al*., 2002). To distinguish AR-mediated ligands from GR-mediated ligands, a known AR antagonist can be added to the assay to block the AR. If receptor binding takes place, it can be attributed to the binding of the GR.

2.4.2.1.4 Oestrogens and the oestrogen receptor (ER)

Oestrogens are important sex steroid hormones produced primarily in the testes and ovaries of males and females, respectively (Lee *et al*., 2012). Oestrogens play a key role in the development of secondary sex characteristics, the reproductive cycle, fertility, maintenance of pregnancy (Legler *et al*., 1999), growth, bone development, and behaviour. The oestrogen steroid hormone oestradiol contributes to energy metabolism and obesity, thereby affecting the balance of glucose and insulin, lipogenesis, and lipolysis (Heindel & Blumberg, 2019). Oestrogens also function as part of the neuroendocrine, cardiovascular, and skeletal systems (Lee *et al*., 2012). Oestrogen signalling pathways are activated or inhibited depending on the activities of the two oestrogen receptors, α-ER and β-ER, which have different downstream transcriptional activities in specific target tissues and organs. The α-ER is expressed in the uterus where it is responsible for uterotrophic responses, in the prostate stroma, ovarian cells, Leydig cells in the testes, epididymis, breast and liver (Lee *et al*., 2012), while the β-ER is found within the prostate epithelium, and ovarian granulosa. Free oestrogens in the bloodstream can diffuse into the cytoplasm of target cells, binding to the oestrogen receptors (Soto *et al*., 2006), followed by heterodimerisation of the hormone-bound ER. This heterodimer binds to the oestrogen response element (ERE) located in the promoter of target genes

(Wilson *et al*., 2004; Kumar *et al*., 2011). During hormone binding to the ER, the ligand binding domain of the receptors undergo conformational changes which allow the recruitment of coactivators (Björnström & Sjöberg, 2005). Specific messenger RNA (mRNA) is translated, leading to the synthesis of the proteins which regulate physiological functions (Wilson *et al*., 2004). 17β-Oestradiol is the dominant oestrogen in the body, with lower levels of oestrone and oestriol present (Björnström & Sjöberg, 2005). Since the ER responds to both natural (e.g., oestrone and E_2) and synthetic oestrogens (e.g., 17α-ethinyl oestradiol, EE_2), oestrogen-like compounds (e.g., bisphenol A and phthalates) (Connon *et al*., 2012) found in the environment are of particular concern (Könemann *et al*., 2018) as they can act as ER agonists or antagonists. Oestrogen receptor transactivation bioassays are one of the most applied *in vitro* reporter gene assays used to screen environmental mixtures for (anti-)oestrogenic activities by evaluating binding to the ER (Mutengwe *et al*., 2016; Könemann *et al*., 2018). One example of such as cell-based system is the hormone-dependent T47D-KB*luc* cell line (Yu *et al*., 2017). This cell line was developed by stably transfecting T47D-KB human breast adenocarcinoma cells, which naturally express the α-ER and β-ER, with an ERE-promoter-luciferase reporter gene construct (Wilson *et al*., 2004).

2.4.2.2 Bioassays indicative of oxidative stress and damage

Apart from xenobiotic metabolism and endocrine disruptive effects, *in vitro* systems can also be applied to measure specific oxidative stress endpoints such as ROS production, antioxidant enzyme activity (e.g., SOD and CAT), and oxidative damage (e.g., LPO) (Connon *et al*., 2012). Oxidative stress responses can be measured in different cell types, including intestinal and liver cells. The GIT is the most common exposure route for xenobiotics, with parent compounds and their metabolites absorbed through the GIT (Ryu *et al*., 2021). Based on this, intestinal cells serve as the first line of defence against xenobiotics, including pesticides. The human epithelial small intestinal HuTu-80 cell line originated from a duodenal adenocarcinoma (Yue *et al*., 2019) and is considered a model for human intestines (Prinsloo *et al*., 2013). Although these cells were initially used to study intestinal cancers (Schmidt *et al*., 1977), HuTu-80 cells also respond to antioxidant compounds, produce hormone-peptides, such as glucagon-like peptide-1 (Yue *et al*., 2019), and have been used to investigate the role of specific genes in oxidative stress-induced apoptosis (Pereira Moreira *et al*., 2009). After the absorption of xenobiotics in the GIT, the liver is the main organ responsible for xenobiotic metabolism, including phase I and II biotransformation reactions which increase the production of ROS (Gu & Manautou, 2012; Ray *et al*., 2012; He *et al*., 2017). The H4IIE rat hepatoma cell line has been well-studied and is known to retain an array of liver functions, including detoxification (Mennillo *et al*., 2019). H4IIE cells contain high levels of antioxidant enzymes (Röhrdanz *et al*., 2002) and have been used to investigate antioxidant responses after exposure to pharmaceuticals (Mennillo *et al*., 2018) and organophosphate esters (Mennillo *et al*., 2019). Based on this, the H4IIE-*luc* and HuTu-80 cell lines are considered suitable models for studying oxidative stress responses elicited by environmental mixtures.

2.4.2.2.1 Intracellular reactive oxygen species

The presence of elevated levels of ROS is a warning sign of oxidative stress (Escher *et al*., 2021). Small-molecule sensors that react to specific stimuli, called fluorescent probes can be used to directly measure the production of ROS, including H_2O_2, in cells. Fluorescent probes are very sensitive and data collection is considered simple (Gomes *et al*., 2005). An example of such a probe is H_2DCF-DA which was first developed by Keston and Brandt (1965). The H_2DCF-DA assay is based on the principle that the membrane-permeable fluorogenic dye diffuses into the cells and is hydrolysed to non-fluorescent 2'-7'dichlorodihydrofluorescein (DCFH) by intracellular esterases where it remains trapped within the cells (LeBel *et al*., 1992). If ROS, such as H_2O_2 and peroxidases, are produced by the cells because of increased oxidative stress, the DCFH will react with the H_2O_2 which oxidises it to a highly fluorescent fluorescein derivative, 2'-7'dichlorofluorescein (DCF) (Wang & Joseph, 1999; Gomes *et al*., 2005). The fluorescent DCF can be quantified using a microplate reader capable of quantifying fluorescence with a specific excitation and emission wavelength (Wang & Joseph, 1999; Katerji *et al*., 2019). The amount of fluorescence is directly proportional to the amount of ROS produced intracellularly (Mattia *et al*., 1991) (**Figure 6**). The ability of the dye to diffuse into cells makes the use of this fluorogenic probe highly suitable for cell-based assays (Gomes *et al*., 2005) and as a result this method has been successfully applied to various *in vitro* models over the years (Lu *et al*., 2012; Chaufan *et al*., 2014; Ilboudo *et al*., 2014; Kašuba *et al*., 2017).

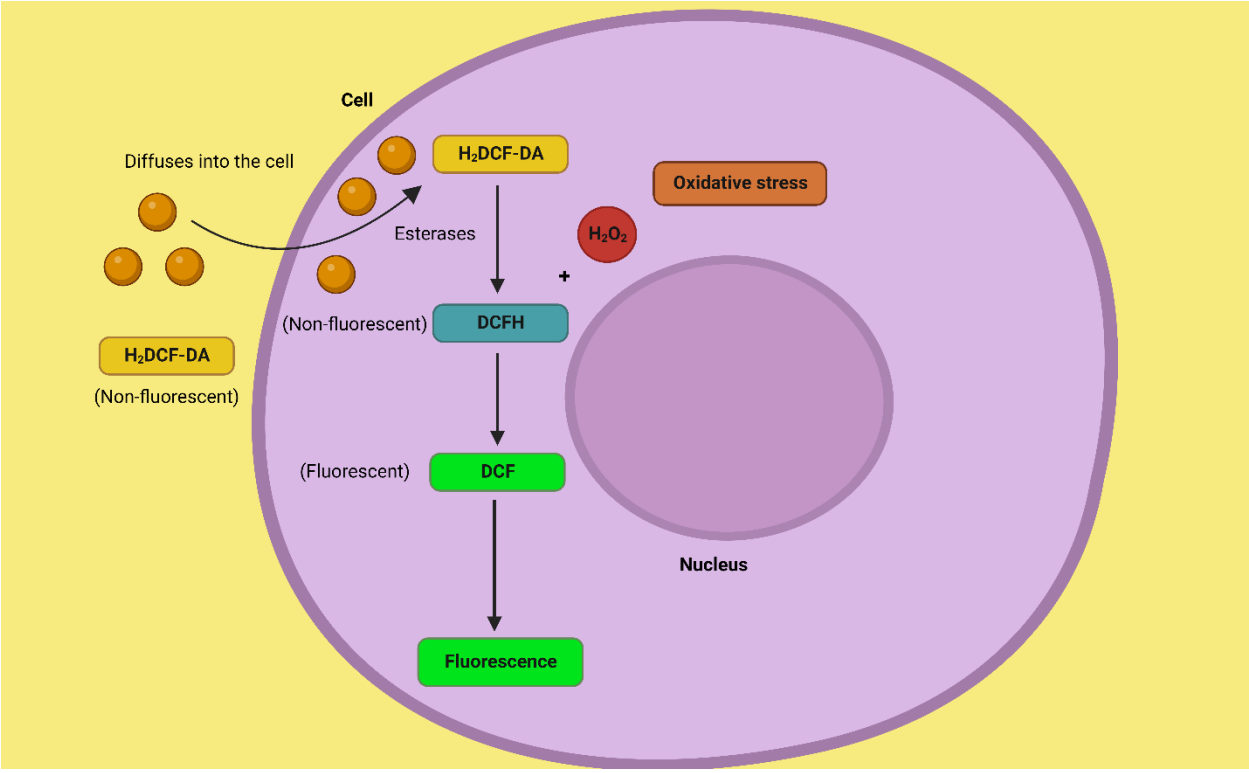

Figure 6 Principle of the 2'-7'dichlorodihydrofluorescein diacetate (H_2DCF-DA) assay used to determine the amount of reactive oxygen species produced inside a cell. DCF: 2'-7'dichlorofluorescein; DCFH: 2'-7'dichlorodihydrofluorescein; H_2O_2: hydrogen peroxide (Wang & Joseph, 1999; Katerji *et al*., 2019) (created in BioRender.com).

2.4.2.2.2 Responses of the superoxide dismutase-catalase system

The activity of antioxidant enzymes, such SOD and CAT, can be measured using *in vitro* cell-based bioassays. Superoxide dismutase counteracts oxidative stress by catalysing the dismutation of $O_2^{\bullet-}$ into H_2O_2 and molecular oxygen (O_2) (McCord & Fridovich, 1969). In the presence of $O_2^{\bullet-}$ certain organic compounds such as pyrogallol spontaneously autoxidise, resulting in a colour change from white to yellow-brown (Roth & Gilbert, 1984; Gray *et al*., 1985). The rate of inhibition of pyrogallol autoxidation can therefore be measured using spectrophotometry (Katerji *et al*., 2019; Singh *et al*., 2020). If SOD is produced intracellularly in response to ROS, white pyrogallol will not autoxidize into a variety of yellow-brown oxidation products. However, a yellow-brown colour indicates autoxidation and no SOD activity (Marklund & Marklund, 1974). The SOD content in cell extracts can be determined by recording the kinetic reaction and quantifying the resulting colour response spectrophotometrically by quantifying optical density at a specified wavelength (Gargouri *et al*., 2020) (**Figure 7**).

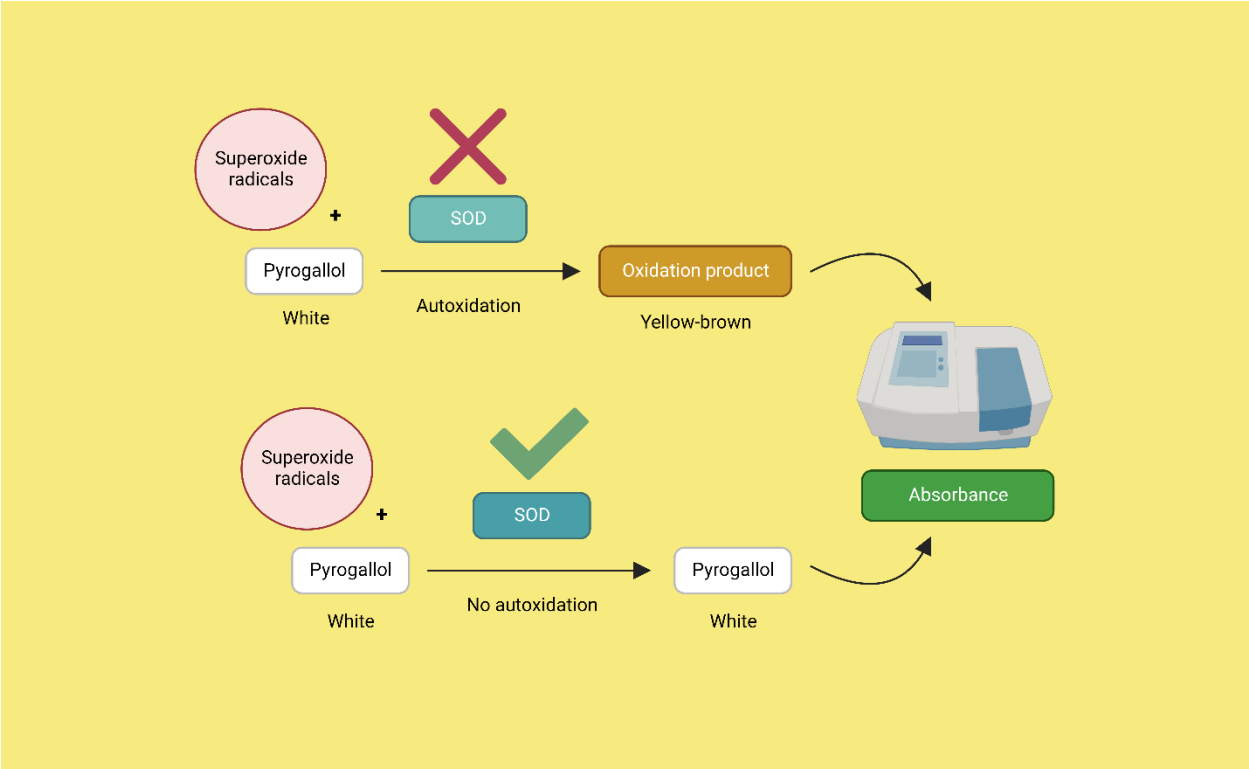

Figure 7 Principle for the determination of superoxide dismutase (SOD) content in cells. In the presence of SOD, superoxide radicals will be converted into molecular oxygen and hydrogen peroxide, and white pyrogallol will not autoxidise into yellow-brown oxidation products. In the absence of SOD, superoxide radicals will not be detoxified and cause the autoxidation of white pyrogallol into yellow-brown oxidation products. In both cases the kinetic reaction is recorded, and the resulting colour response is quantified spectrophotometrically to determine SOD content (Marklund & Marklund, 1974) (created in BioRender.com).

After SOD activity, CAT converts the resulting reactive H_2O_2 into H_2O and O_2 (Chance, 1948). Catalase activity can therefore be determined by measuring the enzyme-catalysed decomposition of H_2O_2 through titration with an excess of a very strong oxidizing reagent, such as potassium permanganate ($KMnO_4$). The reaction is stopped by the addition of sulphuric acid (H_2SO_4) and the resulting colour response is quantified spectrophotometrically. The amount of residual $KMnO_4$ is inversely proportional to the activity of the catalase enzyme (Cohen *et al.*, 1970) (**Figure 8**).

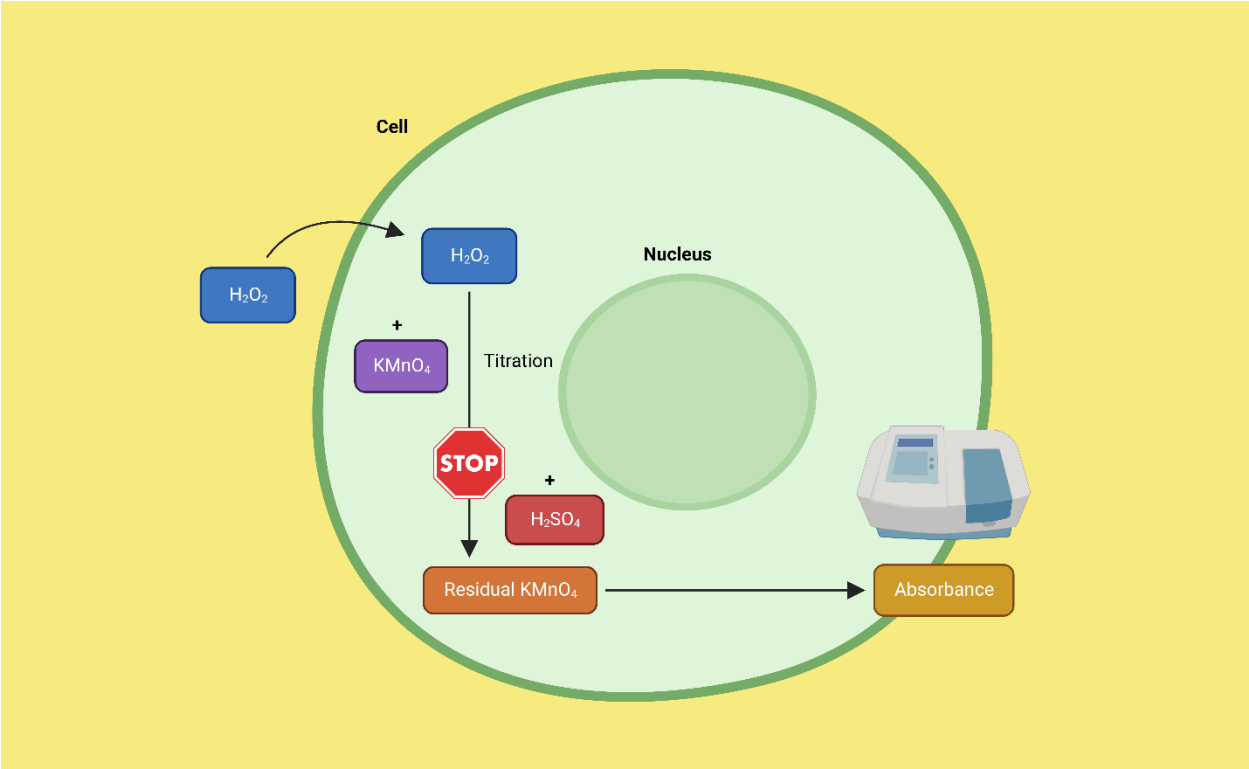

Figure 8 Principle for the determination of catalase (CAT) activity in cells. Cells are stimulated with hydrogen peroxide (H_2O_2) since CAT catalyses the conversion of H_2O_2 into water and molecular oxygen. This enzyme-catalysed decomposition of H_2O_2 is measured through titration with access of potassium permanganate ($KMnO_4$). The reaction is stopped by the addition of sulphuric acid (H_2SO_4) and resulting colour response of the residual $KMnO_4$ is quantified spectrophotometrically (Cohen *et al*., 1970) (created in BioRender.com).

2.4.2.2.3 Oxidative damage in the form of lipid peroxidation

Lipid peroxidation occurs when free radicals bind to a carbon of a fatty acid's alkyl, leading to the oxidative degradation of lipid components within the cellular membranes of an organism. One of the most common methods to determine LPO is the TBARS assay in which malondialdehyde (MDA), one of the end products of LPO, is measured (Ohkawa *et al*., 1979; Boussabbeh *et al*., 2016; Katerji *et al*., 2019). The TBARS assay is a colourimetric assay during which MDA reacts with thiobarbituric acid (TBA) under conditions of high temperature (95–100°C) and acidity. The reaction generates a pink-coloured adduct which can be extracted with an organic solvent (e.g., *n*-butanol) and quantified spectrophotometrically (Almroth *et al*., 2005; Katerji *et al*., 2019) (**Figure 9**). The TBARS assay has been used to evaluate lipid peroxidation in cell lines following pesticide exposure (Ilboudo *et al*., 2014; Kašuba *et al*., 2017).

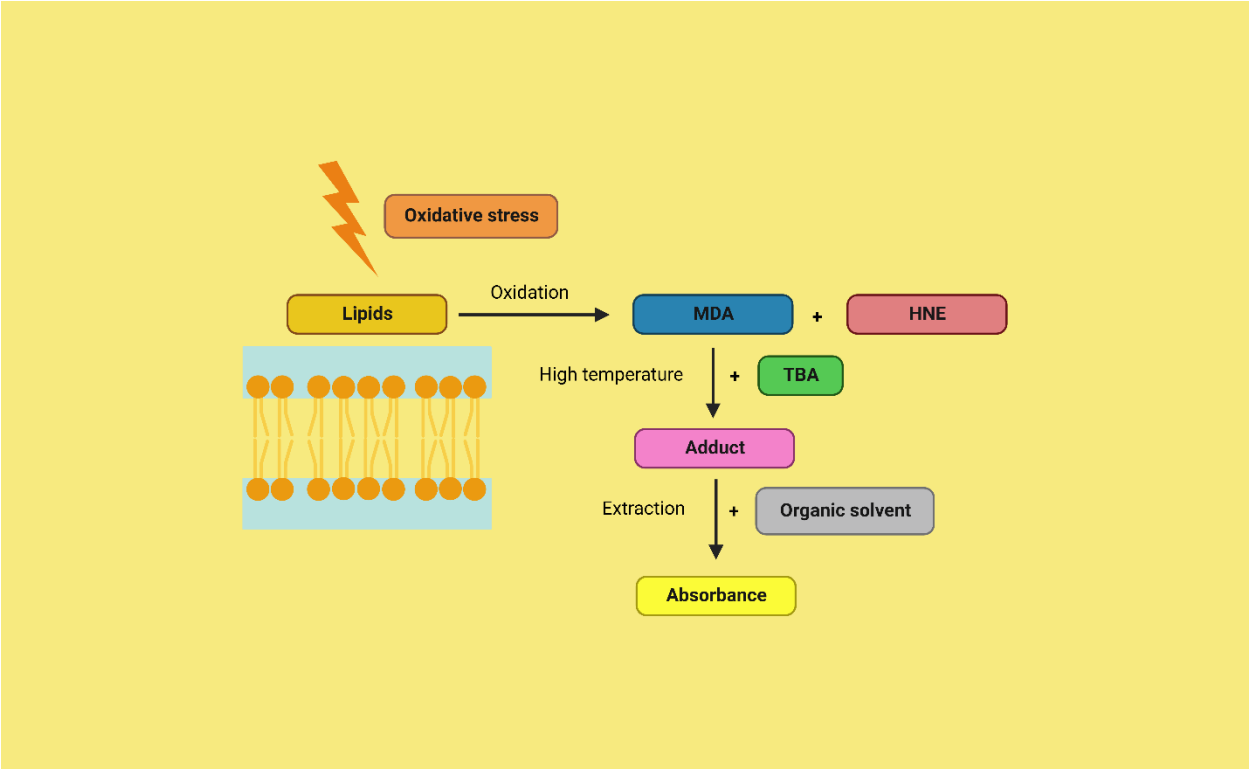

Figure 9 Principle of the thiobarbituric acid reactive substance (TBARS) assay for the determination of lipid peroxidation in cells as malondialdehyde (MDA) content. HNE: 4-hydroxynonenal; TBA: thiobarbituric acid (Ohkawa *et al*., 1979) (created in BioRender.com).

2.4.2.3 Cell-based bioassays to measure acetylcholinesterase activity

As previously mentioned, non-neuronal AChE is produced in liver and intestinal cells. Therefore, AChE activity can be determined *in vitro* using the Ellman assay (Ellman *et al*., 1961). This is a colourimetric assay too based on measuring the colour response when thiocholine reacts with Ellman's reagent [5,5'-dithio-*bis*-(2-nitrobenzoic acid); DTNB] producing yellow 2-nitro-5-thiobenzoate (TNB). A kinetic reaction takes place, and the resulting colour response is quantified spectrophotometrically. The measured absorbance of TNB is proportional to AChE activity (Zimmerman *et al*., 2008; Härtl *et al*., 2011).

2.5 Instrumental chemical analysis to identify compounds present in agrochemical mixtures

Although EBMs provide information about the biological effects of environmental mixtures (Neale e*t al*., 2015; Legradi *et al*., 2018; Brack *et al*., 2019), they do not provide information about the identity of the chemicals present. Consequently, chemical analytical screening can be used to complement EBMs (Brack *et al*., 2019). Using an approach which combines bioassays and instrumental chemical analysis could help identify the chemicals responsible for the observed effects (Escher *et al*., 2020). Suspect screening is one approach which can be followed and describes the use of mass spectrometry data and a list of known chemicals of interest to identify contaminants without prior knowledge of their presence. Suspect screening provides a semi-quantitative analysis of complex environmental mixtures (Escher *et al*., 2020). However,

due to the complexity of environmental mixtures, analytical techniques with high resolving power are required (Ferrer & Thurman, 2003). Higher resolving power also means better selectivity (Lacorte & Fernandez-Alba, 2006). High-resolution mass spectrometry coupled with gas or liquid chromatography enables the screening of many chemicals by detecting thousands of accurate masses associated with unknown chemicals in environmental samples (Brack *et al*., 2018; Escher *et al*., 2020).

One example of such a high-resolution platform is the use of UHPLC-QTOF/MS. Briefly, UHPLC-QTOF/MS is based on the physical separation of individual compounds within a sample between a stationary and mobile phase. Compounds are ionised and separated based on their mass-to-charge ratios (m/z). The mass spectrometer (MS) measures the m/z of the ions and is composed of three basic components: an ion source, an analyser and a detector that counts the ions (Stachniuk & Fornal, 2016). An analyte in a sample can only be measured by an MS if it has been ionised, usually through electrospray ionisation (ESI) (Ferrer & Thurman, 2003). This type of ionisation is also considered ideal for polar compounds, such as pesticides (Hogendoorn *et al*., 1996; Stachniuk & Fornal, 2016). Electrospray ionisation can either result in positively or negatively charged ions (Stachniuk & Fornal, 2016). The widest range of compounds are covered by positive ESI, but it is good practice to extend the coverage by also including negative ESI. Once the analytes have been ionised, they move to the mass analyser for separation.

A quadrupole time-of-flight (Q-TOF) mass analyser combines the benefits of two mass analysers: high compound fragmentation efficiency of quadrupole and the rapid analysis speed and high mass resolution of time-of-flight (Allen & McWhinney, 2019). The first quadrupole acts as a mass filter that selects specific ions based on their m/z, while the second quadrupole serves as the collision cell where the precursor ions are fragmented by bombarding them with neutral gas molecules (e.g., nitrogen). After leaving the quadrupole, the product ions are pulsed by an electric field and accelerated in the ion modulator of the flight tube. Ions with the same kinetic energy enter the 1-m field-free flight tube (Allen & McWhinney, 2019). Time-of-flight (TOF) describes the time it takes an ion with a specific m/z ratio to travel through the flight tube and reaches the detector (Ferrer & Thurman, 2003; Stachniuk & Fornal, 2016). The flight times are proportional to the square root of the m/z of the ion (Lacorte & Fernandez-Alba, 2006), with ions that have a lighter mass travelling faster than ions with a heavier mass. The detector converts the TOF values of the ions into mass signals (Allen & McWhinney, 2019). Since the TOF values are unique for each ion, UHPLC-QTOF/MS can provide accurate mass measurement for parent and fragment ions within 5 ppm (Ferrer & Thurman, 2003; Lacorte & Fernandez-Alba, 2006). Accurate mass measurements also provide the elemental composition of parent and fragment ions (Lacorte & Fernandez-Alba, 2006). The identification of unknown compounds present in complex environmental mixtures entails matching the mass spectrum obtained for a sample with a spectral library of known authentic standards (Ferrer & Thurman, 2003; Allen & McWhinney, 2019).

2.6 Conclusion

Agrochemicals end up in the environment as complex mixtures where they can elicit various biological effects on non-target biota, including xenobiotic metabolism, endocrine disruption, oxidative stress and damage, and AChE inhibition. Although instrumental chemical analysis is often used to quantify compounds present in environmental matrices, it cannot identify all chemicals present and usually requires prior knowledge about the target chemical or chemical class. In addition, instrumental chemical analysis cannot always provide the concentration of chemicals detected, especially if it is below the instrument's limit of detection (LOD) or quantification (LOQ). Since environmental pesticide mixtures contain low concentrations of many individual chemicals, instrumental chemical analysis should be combined with sensitive bioanalytical tools able to measure biological effects that occur at a cellular level. One example is the use of *in vitro* effects-based methods. It is therefore recommended that a combined approach of bioassays to capture the biological effects of the environmental mixture, and quantification of the sample to identify the chemicals likely causing the observed effects should be used to investigate agrochemical mixtures from the South African environment.

CHAPTER 3: MATERIALS AND METHODS

3.1 Experimental setup

The experimental workflow which was followed during this study is illustrated in **Figure 10**. Briefly, agricultural soil was sampled from two sampling locations in South Africa (the Mpumalanga province and Vaalharts Valley, Northern Cape province), followed by the determination of soil properties (total organic carbon content, and soil particle size distribution). The water-soluble (bioavailable) fraction of the soil was extracted and used to prepare soil exposure medium for the respective tissue culture cell lines (H4IIE-*luc*, MDA-kb2, T47D-KB*luc* and HuTu-80). The different cell lines were selected for their capacity to determine various biological endpoints using *in vitro* bioassays. Reporter gene bioassays were used to assess the following receptor-mediated effects: (i) xenobiotic metabolism as AhR agonism (H4IIE-*luc*), and (ii) endocrine disruption as AR and GR agonism, and AR antagonism (MDA-kb2); and ER agonism and antagonism (T47D-KB*luc*). Enzymatic and non-enzymatic cell-based bioassays were used to evaluate (i) oxidative stress responses through ROS production, alterations in antioxidant enzyme activities (SOD and CAT), LPO as MDA content; and (ii) non-neuronal AChE activity. Trial experiments showed that the intestinal (HuTu-80) and liver (H4IIE-*luc*) cells showed better responses in the oxidative stress and AChE bioassays compared to breast cancer cells (MDA-kb2 and T47D-KB*luc*), likely due to their role in detoxification. Consequently, only the HuTu-80 and H4IIE-*luc* cells were selected for investigating oxidative stress responses and AChE activity.

For all bioassays, the cell lines were exposed to the soil extracts using the media replacement method during which the nutrient medium of the cells is removed and replaced with the prepared soil exposure medium (samples). This method is considered suitable to evaluate the biological effects of environmental samples. However, cells were exposed to all reference compounds (positive controls; PCs) and solvent controls (SCs) by directly dosing these chemicals into the nutrient medium of the bioassay, unless specified otherwise. The term ‘dosing’ in the context of this study therefore refers to: transferring a fraction of a chemical to the assay medium to obtain the desired concentration or dose (Fischer *et al*., 2019; Escher *et al*., 2021). The inclusion of SCs in *in vitro* bioassays is essential as it ensures that the solvent itself does not induce a response in the cells (Escher *et al*., 2021). Lastly, the presence and concentration of selected agrochemicals was investigated by chemical quantification and screening of the soil extracts using UHPLC-QTOF/MS. Additional information about all chemicals, reagents, media constituents, consumables, and equipment used during this study can be found in **Table S2**.

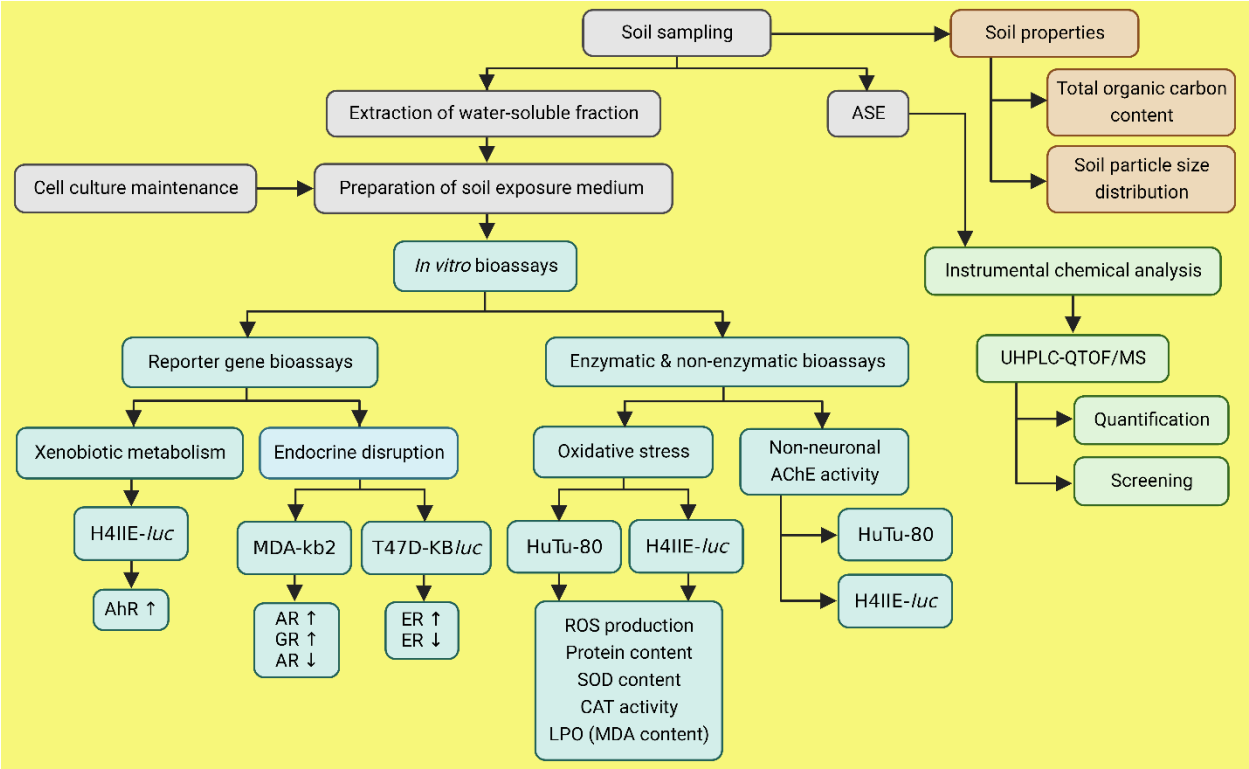

Figure 10 Illustration of the experimental setup followed in this study. ↑: agonism; ↓: antagonism; AChE: acetylcholinesterase; AhR: aryl hydrocarbon receptor; AR: androgen receptor; ASE: accelerated solvent extraction; CAT: catalase; ER: oestrogen receptor; GR: glucocorticoid receptor; UHPLC-QTOF/MS: ultra-high performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry; LPO: lipid peroxidation; MDA: malondialdehyde; ROS: reactive oxygen species; SOD: superoxide dismutase (created in BioRender.com).

3.2 Site descriptions

Two agricultural regions in South Africa that represent some of the highest agricultural inputs of pesticides were selected as the sampling areas—the Mpumalanga province (MP) and the Vaalharts Valley (VH) in the Northern Cape province (Dabrowski, 2015b; Dabrowski, 2015c). Some of the sampling locations in the Highveld escarpment of the MP are within the Upper-Vaal River catchment and Klein Olifants River. The province falls within the grassland biome, with an annual mean rainfall of 800–900 mm (Fourie *et al*., 2017). The area is the largest maize-producing region in South Africa in terms of average yield (1 000 t/ha) (Grain SA, 2021), and to maintain such a high yield, agrochemicals—such as herbicides—are extensively applied. Of the two study areas, this one represents wetter climatic conditions since it has a higher annual rainfall compared to the VH. Consequently, farmers mainly follow rain-fed cultivation practices. There was a total of eleven maize field sampling locations in the MP, stretching from Middelburg in the north along the Klein Olifants-, Life Water- and Vaal Rivers to Standerton in the south (**Figure 11**).

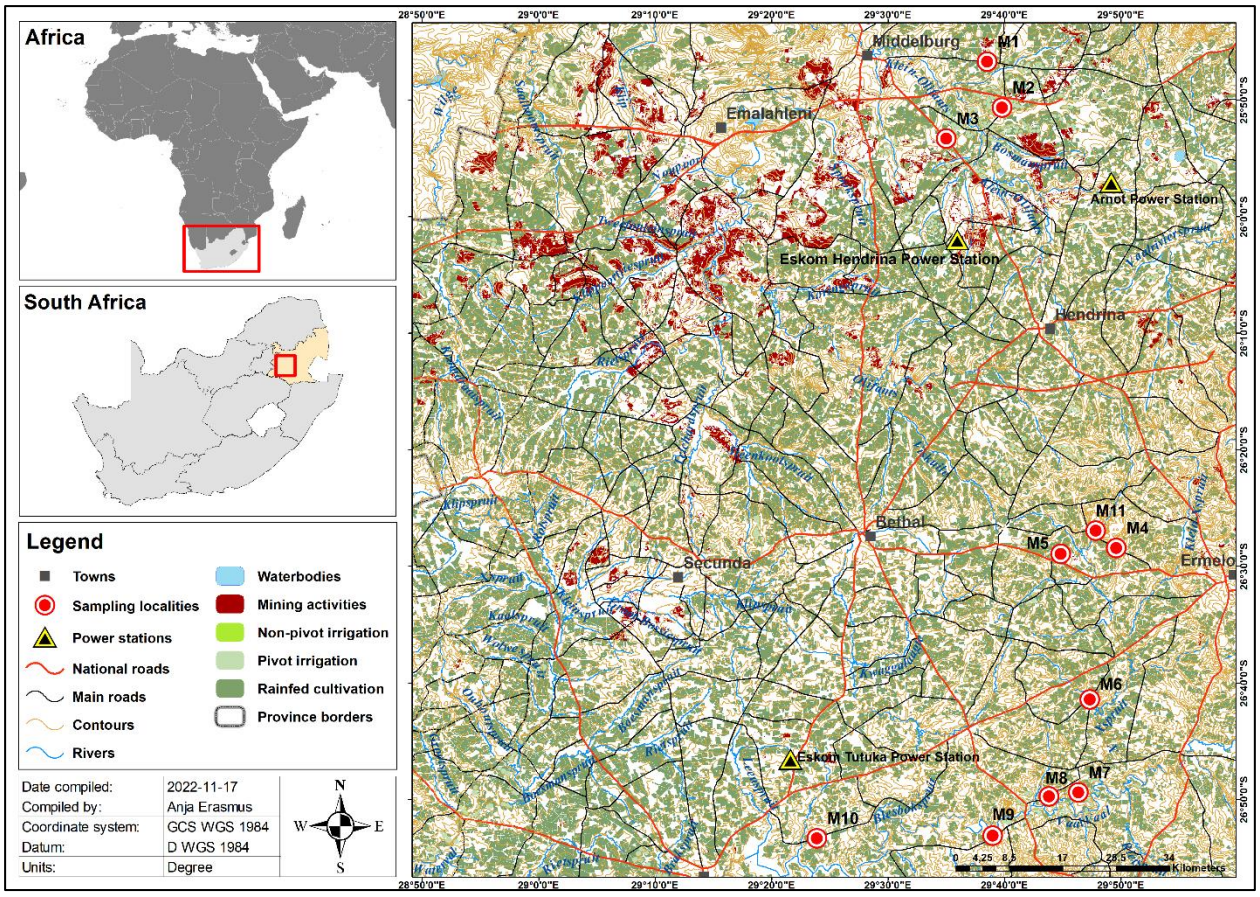

Figure 11 Sampling locations in the Mpumalanga province of South Africa. The red plots indicate the eleven sampling locations; M1–M11: Maize field 1–Maize field 11. There may be some discrepancies between the type of irrigation practices followed as indicated in the above map and those reported in Table S1 since the metadata used in the geographic information system program is from 2017.

The VH is located between the Vaal and Harts Rivers, in the north-east of the Northern Cape province, bordering the North West province of South Africa. The area covers the Vaalharts Irrigation Scheme in which water from a weir in the Lower Vaal River is diverted into a series of channels, siphons, and pipes. This water is used to irrigate crops on more than 1 200 plots (Verwey & Vermeulen, 2011). It is the largest irrigation scheme in the southern hemisphere (Van Vuuren, 2010). Maize is cultivated in the area, but in recent years, pecan nut farming has become more popular. Although the largest part of the Northern Cape province falls within the Nama Karoo biome, the VH falls within the Savanna biome, receiving approximately 400–500 mm of rain annually (Fourie *et al.*, 2017). Due to the drier climatic conditions, many of the farms in the area are irrigated (pivot, and non-pivot irrigation, e.g., drip-irrigation) rather than rain-fed. In the VH there were a total of eight sampling locations, which included four maize fields and four pecan orchards (**Figure 12**). A more detailed description of each sampling location in both the MP and VH can be found in **Table S1**.

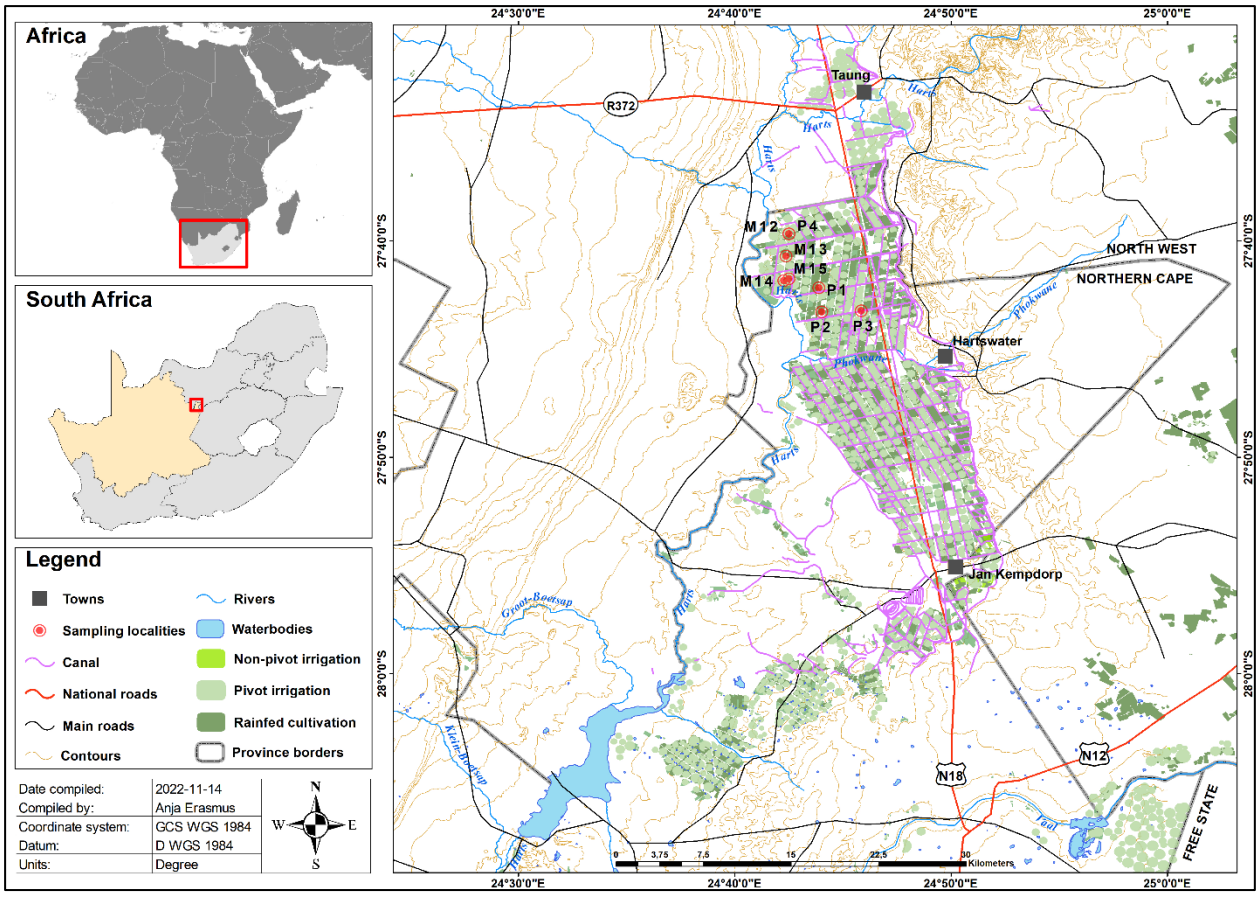

Figure 12 Sampling locations in the Vaalharts Valley in the north-east of the Northern Cape province, bordering the North West province of South Africa. The red plots indicate the eight sampling locations; M12–M15: Maize field 12–Maize field 15; P1–P4: Pecan orchard 1–Pecan orchard 4. There may be some discrepancies between the type of irrigation practices followed as indicated in the above map and those reported in Table S1 since the metadata used in the geographic information system program is from 2017.

3.3 Soil sampling

Composite soil samples were collected in 250 mL ethanol-rinsed, high-density polyethylene (HDPE) bottles (Thermo Scientific™ Nalgene®, AEC-Amersham SOC Ltd) from maize and pecan nut farms in the MP and VH during the period of pesticide application in the maize growing season (November–December of 2020). Soil samples were transported back to the laboratory at 4°C and stored at -20°C until the determination of soil properties and subsequent extraction. All sampling equipment was rinsed with deionised water between sampling locations to avoid contamination.

3.4 Soil properties

Soil properties influence the behaviour of environmental pollutants, such as agrochemicals (Li *et al.*, 2021a). The organic carbon content of soil plays a key role in the partitioning and bioavailability of soil-associated contaminants, especially non-ionic organic chemicals (US EPA, 2001). Soil particle size

(fractions of clay, silt, and sand) influences the binding affinity and bioaccessibility of agrochemicals to soil (Li *et al*., 2021a). The soil properties will therefore affect how readily or not these chemicals can move into receiving water bodies following rainfall or irrigation events.

3.4.1 Total organic carbon

Total organic carbon (TOC) content measures the total amount of oxidisable organic material within the soil. This includes dissolved, particulate, and suspended carbon, as well as colloids (US EPA, 2001). The percentage (%) TOC in soil samples was determined according to the semi-quantitative method of loss-on-ignition, which is based on the heated destruction of all organic matter in the soil (Schumacher, 2002; Gerber *et al*., 2015). Approximately 5 g of soil was transferred to a pre-weighed, acid-washed ceramic crucible, followed by incineration at 600°C for 6 hours in a muffle furnace (Nabertherm GmbH, Labotec (Pty) Ltd). After cooling to room temperature, the crucible was weighed to determine the %TOC as follows:

$$\%TOC = \left[\frac{M_b - M_a}{M_b}\right] \times 100$$

with

%TOC = Total organic carbon content (%)

M_b = Mass before incineration (g)

M_a = Mass after incineration (g)

The %TOC was classified as follows: very low (< 0.05%); low (0.05–1%); moderately low (1–2%); medium (2–4%); and high (> 4%) (Gerber *et al*., 2015).

3.4.2 Soil particle size distribution

The soil type of the different sampling locations was described based on soil particle size distribution (% composition) as determined according to the Endecott dry-sieving method described by Gerber *et al*. (2015). Briefly, Endecott sieves with different mesh sizes (53, 212, 500, 2 000 and 4 000 µm) were stacked on top of one another, from the largest (4 000 µm) to the smallest (53 µm) mesh size, with a final collection pan at the bottom. After the soil had been dried over-night at 35°C in an oven, 20 g of soil (total mass) was manually sieved for 10 minutes to collect the following soil particle fractions: clay (< 53 µm); fine sand (53–212 µm); medium sand (212–500 µm); coarse sand (500–2 000 µm); very coarse sand (2 000–4 000 µm); and gravel (> 4 000 µm). The soil retained in each sieve was weighed (mass of fraction) and the soil particle size distribution (% composition) was calculated as follows:

$$\% \text{ Composition} = \frac{\text{Mass of fraction (g)}}{\text{Total mass (g)}} \text{ x } 100$$

3.5 Extraction of water-soluble fraction from soil

Since this study aimed to investigate the effects associated with water-soluble agrochemicals, it was important to mimic environmental conditions—the movement of agrochemicals from the point of application (i.e., the soil) towards non-target areas (e.g., water sources) following irrigation or rainfall (Horn *et al*., 2019). Consequently, water was used as the extraction medium to extract target compounds from the soil which would be bioavailable to non-target biota, rather than organic solvents which force compounds not naturally bioavailable in the environment, into solution. Since pesticides—including atrazine which is commonly applied to maize crops—have been detected in rainwater (Van Maanen *et al*., 2001), harvested rainwater was not used as the extraction medium as it could result in false positive results. Rather, compounds were extracted with ultrapure deionised water (18.2 MΩ-cm) from an in-house ELGA water purification system (PURELAB® Ultra MK2 version 3 11/08, Labotec (Pty) Ltd). Based on the acidity of rainfall in each of the sampling areas, pH-adjusted water was used. For the MP and VH soil samples, deionised water with a pH of 4.3 and 7, respectively, was used. The MP sampling area had the low pH because of air pollution caused by the coal-fired power stations but the VH area is far removed from such pollution (Conradie *et al*., 2016; Simpson *et al*., 2019). Water-soluble compounds were extracted from the soil following the method described by Horn *et al*. (2020) (**Figure 13**). Briefly, 20 mL of deionised water was added to 10 g of soil in a 50 mL centrifuge tube (Falcon®, Lasec SA (Pty) Ltd) and shaken at 150 rpm for 1 hour on a mechanical shaker (Labcon® SP015+UPF75, Labcon Laboratory Equipment (Pty) Ltd); where after the soil and water mixture was centrifuged (Hermle Z 32 HK, Labortechnik GmbH, Lasec (Pty) Ltd) at 3 000 *g* for 20 minutes. This process was repeated twice, and the aqueous supernatants were pooled.

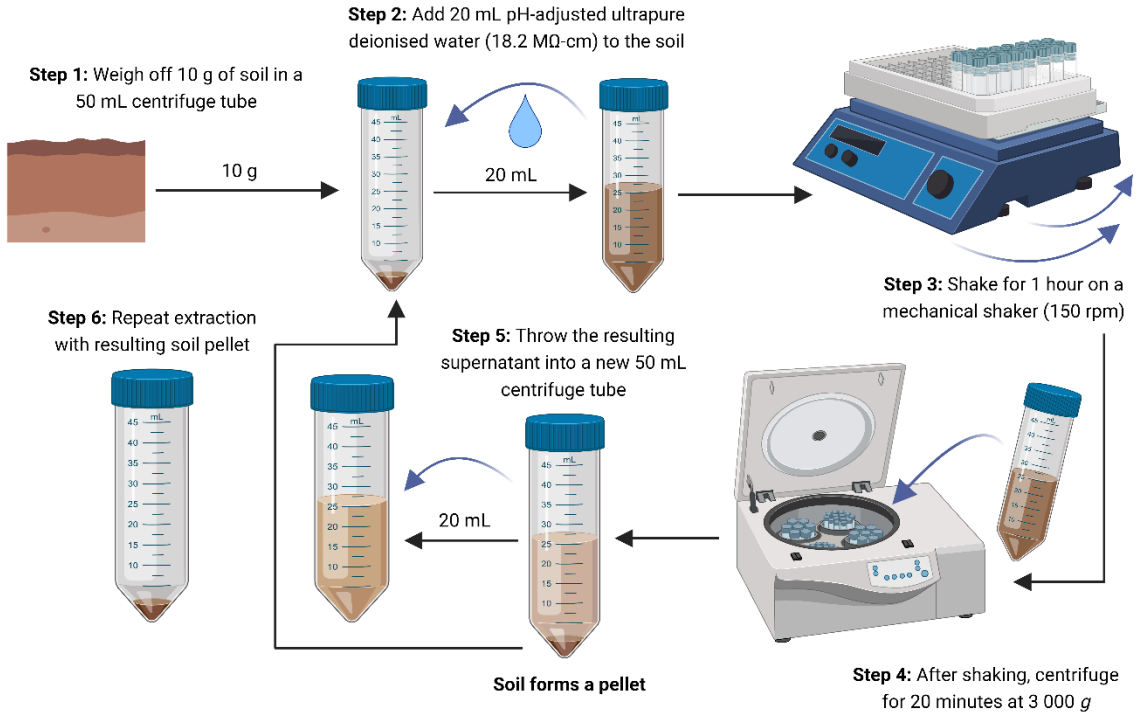

Figure 13 Procedure for extracting the water-soluble fraction (bioavailable) from the soil (Horn *et al*., 2020). *g*: gravitational force; rpm: revolutions per minute (created in BioRender.com).

3.6 Preparation of soil exposure medium for all bioassays

During all bioassays the cells were exposed to the bioavailable fraction of the soil by using the soil extracts (Section 3.5) to prepare nutrient medium—called soil exposure medium (**Figure 14**). Soil exposure media was prepared by adding 20 mL of the supernatant from the soil extraction procedure to each cell line's powdered nutrient medium in a 50 mL centrifuge tube together with the supplements listed in **Table 1** (Horn *et al*., 2020). This procedure was followed for the H4IIE-*luc*, MDA-kb2 and T47D-KB*luc* cells when binding to their respective receptors were investigated.

Foetal bovine serum (FBS) contains growth factors, cytokines, and steroid hormones (e.g., oestradiol and corticosterone) (Dang & Lowik, 2005; Mesalam *et al*., 2017). Since the luminescence reporter gene bioassays were used for evaluating endocrine disruptive endpoints, endogenous hormones had to be removed from the FBS (Dang & Lowik, 2005) to avoid any false positive results. To prepare the hormone-free soil exposure medium, the nutrient medium was supplemented with 10% FBS stripped from its hormones in the place of the usual FBS. This hormone-stripped FBS was prepared by heating FBS in a water bath (Labcon®, Labdesign Engineering (Pty) Ltd) at 56°C for 30 minutes (Nimura *et al*., 2010). The heat-inactivated FBS was added to a 50 mL centrifuge tube containing dextran coated charcoal (165:1; *v/w*) (Sigma®, Sigma-Aldrich (Pty) Ltd) (Murate *et al*., 1989), followed by incubation in a water bath for 45 minutes at 45°C, and centrifugation at 1 000 *g* for 20 minutes. This process was repeated twice, and the

resulting charcoal dextran treated (CDT) FBS was sterile filtered using a Nalgene® 0.22 µm polyethersulfone bottle-top filter (AEC-Amersham SOC Ltd) (Dang & Lowik, 2005). This procedure was followed for the H4IIE-*luc* and MDA-kb2 cell lines, yielding a soil exposure medium supplemented with 10% CDT FBS. However, due to the ER's sensitivity to xenoestrogens and any other additional hormones, the nutrient medium of the T47D-KB*luc* cell line was supplemented with a commercial CDT FBS from HyClone™ (Cytiva, Promolab (Pty) Ltd T/A Separations). This protocol required only a 5% supplementation with CDT FBS. This was done to ensure the removal of any additional hormones that could potentially interfere with the outcome of the T47D-KB*luc* bioassay, whilst still proving the cells with enough nutrients for growth. The soil exposure media was sterile filtered in a Class II biosafety cabinet (BiofFlow-II, Labotec (Pty) Ltd) using a 10 mL syringe (Surgi Plus, Symbiolab (Pty) Ltd) fitted with a 0.22 µm syringe filter (Pall Acrodisc®, Promolab (Pty) Ltd T/A Separations). The resulting soil exposure media were serially diluted (1:2) to obtain the following exposure concentrations: 1, 3, 9, 28, 83 and 250 mg/mL. All three cell lines were exposed to the soil extracts via the media replacement method (previously explained).

During the bioassays that assess oxidative stress responses and AChE activity in the HuTu-80 and H4IIE-*luc* cell lines, the soil exposure medium was prepared in the same manner as described above for the luminescence reporter gene bioassays, with a few slight modifications: The HuTu-80 and H4IIE-*luc* cells were cultivated in nutrient medium as described in **Table 1** containing hormone-rich FBS. Since the oxidative stress responses and AChE activity do not measure hormone-dependent nuclear receptor endpoints, it was not necessary to prepare hormone-free soil exposure medium. Rather, the nutrient medium was supplemented with 10% FBS. The resulting soil exposure media were also sterile filtered and the HuTu-80 and H4IIE-*luc* cells were exposed to 28 mg/mL when assessing oxidative stress responses and AChE activity via the media replacement method. From here on the prepared soil exposure media will simply be referred to as "the samples".

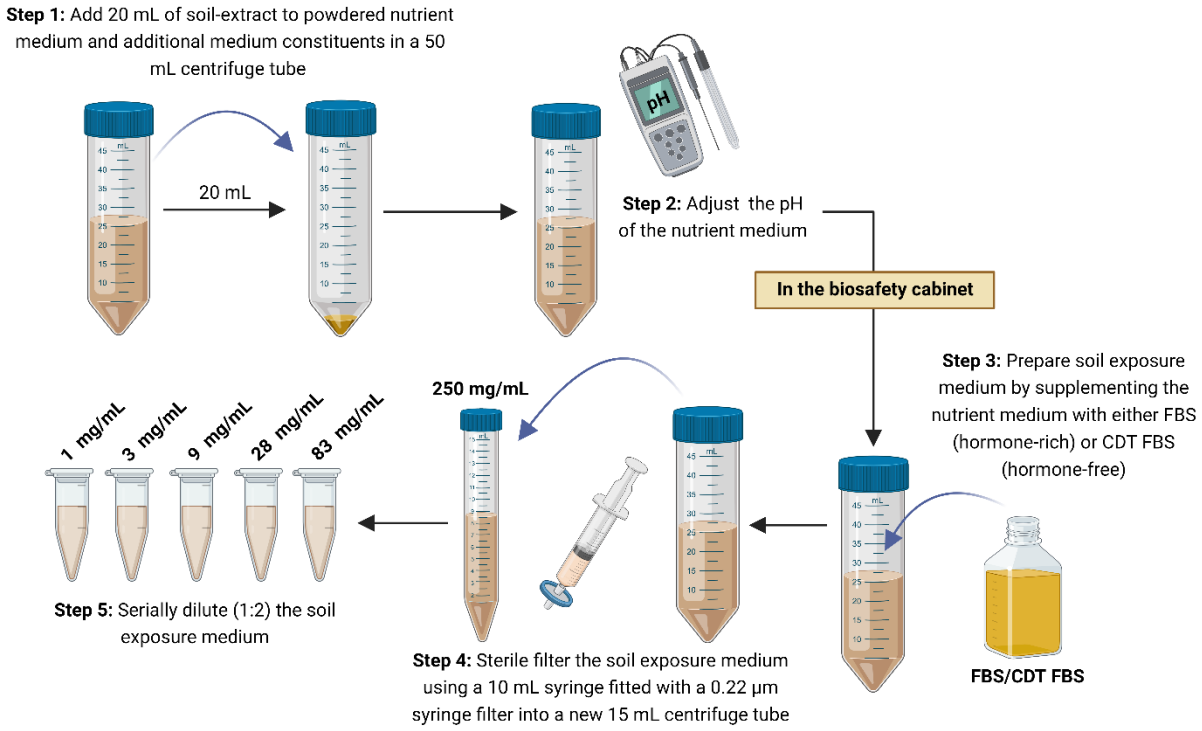

Figure 14 Preparation of soil exposure media (samples) for all bioassays using soil extracts. CDT FBS: charcoal dextran treated foetal bovine serum; FBS: foetal bovine serum (created in BioRender.com).

3.7 *In vitro* bioassays to determine biological effects of agricultural soils

3.7.1 Cell culture maintenance

The H4IIE-*luc* and MDA-kb2 cells were gifted by the University of Saskatchewan (Saskatchewan, Canada), while the T47D-KB*luc* and HuTu-80 cell lines were acquired from the American Type Culture Collection (Virginia, United States of America). Cells were initiated from cryopreservation (liquid nitrogen) by adding 1 mL of thawed cells to 11 mL of their respective nutrient medium (**Table 1**) in a 15 mL centrifuge tube (Falcon®, Lasec (Pty) Ltd), followed by centrifugation at 125 *g* for 10 minutes to form a cell pellet. The resulting supernatant was discarded, and the cells were re-suspended in nutrient medium and transferred to a 60.1 cm^2 tissue culture dish (TPP, Promolab (Pty) Ltd T/A Separations) using a 10 mL serological pipette (Jet Biofil®, Symbiolab (Pty) Ltd). This was done for the H4IIE-*luc*, MDA-kb2 and HuTu-80 cells. The same procedure was followed for T47D-KB*luc* cell line with the exception that Corning® 15 mL centrifuge tubes and 60.1 cm^2 tissue culture dishes (The Scientific Group (Pty) Ltd), and Stripette™ 10 mL serological pipettes (The Scientific Group (Pty) Ltd) were used. This exception was made because these specific consumables show minimal interference with the ER and do not affect the outcome of the T47D-KB*luc* bioassay: the type of plastic the consumable consists of can directly affect the response of the cells since some plasticware contain xenoestrogens that can interfere with the ER (Escher *et al*., 2021). All the cell lines used in this study are immortalised and adherent in nature.

Table 1 Growth conditions of each specific cell line.

Cell line	Nutrient medium	pH	Temperature	CO_2
H4IIE-*luc*	DMEM with low glucose and without phenol red, 3.7 g/L $NaHCO_3$, 10% FBS	7.1	37°C	5%
HuTu-80	DMEM with low glucose and without phenol red, 3.7 g/L $NaHCO_3$, 10% FBS, 1% antibiotic-antimycotic mixture	7.1	37°C	5%
MDA-kb2	Leibovitz (L-15) medium containing L-glutamine, 10% FBS, 1% antibiotic-antimycotic mixture	7.3	37°C	0%
T47D-KB*luc*	RPMI-1640 medium, 2.5 g/L D(+)-glucose, 1.5 g/L $NaHCO_3$, 100 mM sodium pyruvate solution, 1 M HEPES buffer, 10% FBS	7.3	37°C	5%

CO_2: carbon dioxide; DMEM: Dulbecco's Modified Eagle's Medium; FBS: foetal bovine serum; HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; $NaHCO_3$: sodium bicarbonate; RPMI-1640: Roswell Park Memorial Institute-1640.

Prior to the experiments, each cell line was routinely cultured under sterile conditions in tissue culture dishes in their respective nutrient medium (**Table 1**). Briefly, H4IIE-*luc* and HuTu-80 cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) (Sigma®, Sigma-Aldrich (Pty) Ltd) with low glucose and without phenol red supplemented with 3.7 g/L sodium bicarbonate ($NaHCO_3$) (CAS #144-55-8, Sigma®, Sigma-Aldrich (Pty) Ltd), and 10% FBS (Capricorn Scientific, Biocom Africa (Pty) Ltd). The HuTu-80 cells were routinely cultured with 1% antibiotic-antimycotic mixture (Biowest, Celtic Molecular Diagnostics (Pty) Ltd) since the GIT is naturally colonised by bacteria that fulfil important physiological functions (Coleman & Haller, 2018). Therefore, cultivation of the HuTu-80 cells under laboratory conditions required the use of antibiotics. MDA-kb2 cells were cultured in Leibovitz (L-15) medium containing L-glutamine (Sigma®, Sigma-Aldrich (Pty) Ltd) and supplemented with 10% FBS. MDA-kb2 cells were also routinely cultured with 1% antibiotic-antimycotic mixture. In the case of the MDA-kb2 cells, antibiotics were used to combat persistent bacterial infection. The use of antibiotics during culture maintenance is not ideal since it has been found to interfere with the outcome of a bioassay. However, previous experiments in our laboratory showed that the use of antibiotics did not affect binding to the AR. Lastly, T47D-KB*luc* cells were grown in Rosswell Park Memorial Institute-1640 (RPMI-1640) medium (Sigma®, Sigma-Aldrich (Pty) Ltd) supplemented with 2.5 g/L D(+)-glucose (CAS #50-99-7, Sigma®, Sigma-Aldrich (Pty) Ltd), 1.5 g/L $NaHCO_3$, 100 mM sodium pyruvate solution (CAS #113-24-6, Sigma®, Sigma-Aldrich (Pty) Ltd), 1 M HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] buffer (Corning®, The Scientific Group (Pty) Ltd), and 10% FBS. The pH of the nutrient media was adjusted as follows: H4IIE-*luc* and HuTu 80—pH 7.1; MDA-kb2 and T47D-KB*luc*—pH 7.3 (**Table 1**). All cells were maintained in humidified air supplemented with 5% carbon dioxide (CO_2) (except the MDA-kb2 cells) at 37°C. All the water-jacketed incubators were fitted with high efficiency particulate air (HEPA)

filters (Thermo Scientific™, Labotec SA (Pty) Ltd) and in-line filters (Sartorius, Midisart® 2000, Labotec (Pty) Ltd) to prevent bacterial and fungal contamination.

The nutrient media of the cells were replaced every two to five days and when tissue culture dishes were 80–100% confluent, the cells were sub-cultured by passaging them into new tissue culture dishes. Passaging entailed removing the nutrient medium from the tissue culture dishes using a 10 mL serological pipette and gently washing the cells three times with 5 mL Dulbecco's phosphate-buffered saline (DPBS; pH 7.4) (Sigma®, Sigma-Aldrich (Pty) Ltd) using a 3 mL plastic Pasteur pipette (Labocare™, SymbioLab (Pty) Ltd). Cells were trypsinised: detached from the tissue culture dish by the addition of 1.5 mL trypsin-ethylenediaminetetraacetic acid (EDTA) disodium salt (Biowest, Celtic Molecular Diagnostics (Pty) Ltd) and incubated for 3 minutes at 37°C. After removing the trypsin, the cells were carefully harvested and re-suspended in nutrient medium and transferred to a new tissue culture dish. Cells were sub-cultured for a minimum of three passages before performing *in vitro* bioassays.

3.7.2 Determining seeding density prior to any cell-based bioassay

The cell density at which the various cell assays were done, are reported in each instance. This cell density was determined using an automated system. After trypsinising (as previously described) the cells were carefully harvested, transferred to a 15 mL centrifuge tube, and counted according to the trypan blue exclusion method. Trypan blue exclusion is based on the principle that viable cells have intact cell membranes which do not allow (i.e., exclude) the penetration of certain dyes, such as trypan blue, into the cell cytoplasm. Therefore, viable cells will have a clear cytoplasm, whereas non-viable cells will have a blue cytoplasm (Strober, 2015). Briefly, 100 µL of 0.4% trypan blue solution (CAS #72-57-1, Sigma-Aldrich (Pty) Ltd) was added to 100 µL of cell suspension (1:1), followed by loading 10–12 µL of the cell-trypan blue mixture into a reusable cell counting slide (LUNA™, Logos Biosystems Inc). Cells were counted using an automated bright-field cell counter (LUNA-II™, Logos Biosystems Inc).

3.7.3 MTT cell viability assay

In this assay, cell viability is presented as a measure of the cell's ability to reduce tetrazolium salts e.g. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Berridge *et al*., 2005). Since MTT reduction is specifically associated with mitochondrial function, the MTT assay therefore measures the activity of mitochondrial enzymes, such as succinate dehydrogenase (Mosmann, 1983). It is a colourimetric assay in which yellow-coloured MTT is reduced to its purple-coloured formazan through enzymatic reactions and the coenzyme nicotinamide adenine dinucleotide + hydrogen (NADH) that cleaves the tetrazole ring (Grela *et al*., 2018). Viable cells metabolise the MTT, resulting in a colour change (from yellow to purple) which can be quantified spectrophotometrically by measuring absorbance (Mosmann, 1983) (**Figure 15**). The MTT assay was used to i) screen the soil samples for cytotoxicity, ii) verify the

results obtained during the reporter gene bioassays (Section 3.7.4), and iii) determine the highest non-cytotoxic concentration to be used during the enzymatic and non-enzymatic bioassays (Section 3.7.5).

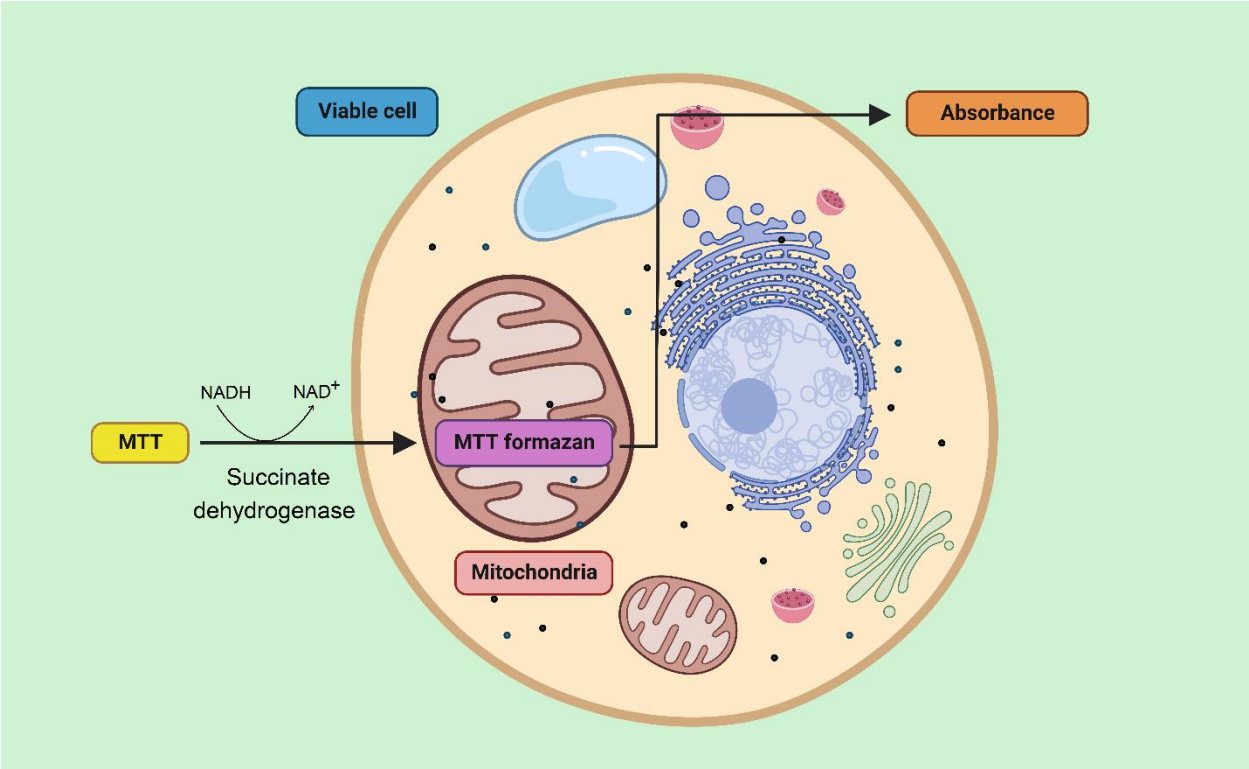

Figure 15 A simplified representation of the MTT cell viability assay's principle. MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NADH: nicotinamide adenine dinucleotide + hydrogen (Mosmann, 1983; Grela *et al*., 2018; Patpan *et al*., 2019) (created in BioRender.com).

At the beginning of the MTT assay, cells were seeded (seeding volume = 250 µL/well) in 96-well, clear, flat bottom cell culture test plates (TPP, Promolab Pty Ltd T/A Separations) at the following seeding densities: 80 000 cells/mL (H4IIE-*luc* and HuTu-80), 120 000 cells/mL (MDA-kb2) and 100 000 cells/mL (T47D-KB*luc*). Cells were seeded in the inner 60 wells, while the outer 36 wells were filled with 250 µL DPBS to create a constant micro-environment, avoiding media evaporation and any edge effect. On the last day of the assay, the cells were visually inspected under an inverted phase-contrast microscope (Nikon Eclipse TS100-F, Nikon Instruments Inc, United States of America) for any sign of microbial contamination. Confluency of the cells in each well was also noted. For the MTT assay, a negative control (i.e., cells experiencing severe toxicity) was included by replacing the media from column 12 of the 96-well plate with 200 µL of methanol. This was followed by washing the entire plate three times with DPBS supplemented with Ca^{2+} as calcium chloride ($CaCl_2$) (CAS #10043-52-4, Sigma®, Sigma-Aldrich (Pty) Ltd) and Mg^{2+} as magnesium sulphate heptahydrate ($MgSO_4.7H_2O$) (CAS #10034-99-8, Sigma®, Sigma-Aldrich (Pty) Ltd), after which 100 µL of the MTT solution (0.5 mg/mL) (Sigma®, Sigma-Aldrich (Pty) Ltd) prepared in nutrient medium (without FBS) was added to each well. Cells were incubated for 2 hours at 37°C to ensure that the living cells metabolise the MTT. After incubation, the cells were inspected for

the formation of purple formazan crystals under a microscope. The formed crystals were dissolved in 200 µL of dimethyl sulphoxide (DMSO) (CAS #67-68-5, Chromosalv® Plus, Honeywell Riedel de Haën™, Sigma-Aldrich (Pty) Ltd) and incubated at room temperature in the dark for 30 minutes. The optical density of the solubilised formazan crystals was quantified spectrophotometrically at 560 nm using a multimode microplate reader (TriStar LB 941, Berthold Technologies, Germany). Cell viability of the exposed cells was expressed as a percentage of absorbance compared to the cells treated with only the solvent (SC) of each respective assay.

Sample concentrations that were overall not cytotoxic towards the respective cell lines were used for the subsequent luminescence reporter gene (1, 3, 9, 28, 83 and 250 mg/mL), oxidative stress (83 mg/mL) and AChE bioassays (83 mg/mL). During the assessment of the xenobiotic metabolism (Section 3.7.4.1) and endocrine disruptive potential (Section 3.7.4.2) of agrochemicals related to maize and pecan soil, the MTT cell viability assay was also run in parallel to the luminescence reporter gene bioassays for quality control, i.e., to prevent false negative luminescence reporter gene assay results. It is important that reporter gene bioassays are accompanied by cytotoxicity assessment since cytotoxicity can mask the effects of receptor-mediated bioassays (Escher *et al*., 2021).

3.7.3.1 Data calculation for MTT cell viability assay

The cell viability was determined using Microsoft Excel®. Briefly, the mean, and standard deviation were calculated. The mean absorbance of the negative control (NC) (i.e., non-viable cells) were subtracted from each individual absorbance measurement and expressed in terms of the SC as a percentage. The cell viability (%) was calculated as follows:

$$\%\text{Cell viability} = \left(\frac{\text{ABS}_\text{S} - \text{Mean ABS}_\text{NC}}{\text{Mean ABS}_\text{SC}}\right) \text{x } 100$$

with

ABS_S = Absorbance of cells exposed to the sample

$\text{Mean ABS}_\text{NC}$ = Mean absorbance of negative control

$\text{Mean ABS}_\text{SC}$ = Mean absorbance of the solvent control

The effect of the samples on cell viability was interpreted as follows: non-toxic (> 80%); weakly cytotoxic (60–80%); moderately cytotoxic (40–60%); and strongly cytotoxic (< 40%) (ISO, 2009; López-García *et al*., 2014). Statistically significant ($^*p \leq 0.05$) differences in cell viability between the treated and untreated cells were determined using the Statistical Package for Social Sciences (SPSS) version 24 (IBM). First, the Kolmogorov-Smirnov test was used to determine whether the data was normally distributed. However,

since the data was not normally distributed, the non-parametric Mann-Whitney *U* test was used with a confidence interval of 95%. All data is presented as mean cell viability ± standard deviation (%). Next, the Kruskal-Wallis H test was used to determine whether there were any statistically significant differences in cell viability between the respective cell lines (i.e., H4IIE-*luc*, MDA-kb2, T47D-KB*luc*, and HuTu-80). However, the Kruskal-Wallis H test can only be used to determine whether there are any statistically significant differences between multiple groups, it cannot distinguish which specific pairs are significantly different. Therefore, the Mann-Whitney *U* test was used to determine between which cell lines the cell viability was statistically significant. Due to the multiple groups involved, this approach often leads to Type 1 errors (an overestimation of the number of statistically significant differences). Consequently, Holm's sequential Bonferroni was used to adjust the target *p*-value (i.e., 0.05) as follows (Holm, 1979):

$$\text{Adjusted } p - \text{value} = \frac{(\text{Target } p - \text{value})}{(\text{n} - \text{rank number of pairs in terms of degree of significance}) + 1}$$

With

Target *p*-value = 0.05

n = Number of pairs

3.7.4 Reporter gene bioassays

Cell-based reporter gene bioassays detect effects mediated through receptors that belong to the nuclear receptor superfamily of transcription factors, including non-steroidal nuclear receptors (e.g., AhR) and steroidal nuclear receptors (e.g., ER, AR, and GR) (Sonneveld *et al*., 2006; Connon *et al*., 2012; Timmermans *et al*., 2019). Reporter gene bioassays make use of cells that have been stably transfected with a plasmid that contains multiple copies of the response element of a target receptor, followed by multiple reporter genes (Escher *et al*., 2020). These reporter genes encode easily measurable proteins (Escher e*t al*., 2020). As previously mentioned, the most common reporter gene used is the *luc* gene which expresses luciferase (a luminescent enzyme) (Sonneveld *et al*., 2006). Upon receptor binding and in the presence of the substrate (luciferin), the *luc* gene is expressed and the amount of light emitted (luminescence) is quantified spectrophotometrically (Legler *et al*., 1999; Denison *et al*., 2004).

When the cells were at their third passage, the *in vitro* luminescence reporter gene bioassays were conducted. The overall approach for all the luminescence reporter gene bioassays was as follow: On day one, cells were seeded in the inner 66 wells, while the outer 30 wells were filled with 250 µL DPBS to create a constant micro-environment, avoiding media evaporation and any edge effect. After 24 (H4IIE-*luc* and T47D-KB*luc*) or 48 hours (MDA-kb2) of incubation, the respective cell lines were exposed to the samples and reference compounds (as PCs) for 24 (T47D-KB*luc*), 48 (MDA-kb2), or 72 hours (H4IIE-*luc*).

The samples and reference compounds were serially diluted so that a concentration response curve was obtained. Each concentration was dosed in triplicate. Blank controls (BCs) and SCs were also included (**Figure 16**). The BCs consisted of untreated cells, while the SCs comprised cells which had been exposed to the solvent for each respective assay.

On the last day of the assay and after the cells had been exposed to the samples and reference compounds, cells were microscopically inspected for any sign of bacterial or fungal contamination, or cytotoxicity in the form of cell detachment, vacuolisation, or membrane degradation (Wilson *et al*., 2002). Confluency of the cells in each well was also noted. The nutrient medium containing the samples was removed and the cells were washed three times with DPBS (containing Ca^{2+} and Mg^{2+}), followed by the addition of 25 µL reporter lysis buffer and rapid freezing at -80°C. After one freeze-thaw cycle, luciferase activity was determined by quantifying luminescence using a Berthold multimode microplate reader which automatically injects 100 µL of a luciferase assay reagent (LAR) [20 mM tricine (CAS #5704-04-1, Sigma®, Sigma-Aldrich (Pty) Ltd); 1.07 mM magnesium carbonate hydroxide pentahydrate ($(MgCO_3)_4Mg(OH)_2.5H_2O$) (CAS #56378-72-4, Sigma®, Sigma-Aldrich (Pty) Ltd); 2.67 mM $MgSO_4.7H_2O$ (CAS #10034-99-8, Sigma®, Sigma-Aldrich (Pty) Ltd); 0.1 mM EDTA (CAS #6381-92-6, Sigma®, Sigma-Aldrich (Pty) Ltd); 33.3 mM dithiolthreitol (DTT) (CAS #3483-12-3, Melford, Promolab Pty Ltd T/A Separations); 270 µM coenzyme A (CAS #85-61-0, Melford, Promolab Pty Ltd T/A Separations); 530 µM adenosine-5'-triphosphate (ATP) disodium salt trihydrate (CAS #51963-61-2, Melford, Promolab Pty Ltd T/A Separations); and 470 µM beetle luciferin, potassium salt (Promega, Anatech Instruments (Pty) Ltd)] (Villeneuve *et al*., 1999). Upon injection of the LAR, the luciferin substrate and appropriate co-factors in the LAR are utilised, resulting in the emission of light which is expressed as relative light units (RLUs). The amount of light emitted (luciferase activity) is proportional to the number of ligands (pollutants/agrochemicals) present within the soil extracts that bind to the receptor (Denison *et al*., 2004).

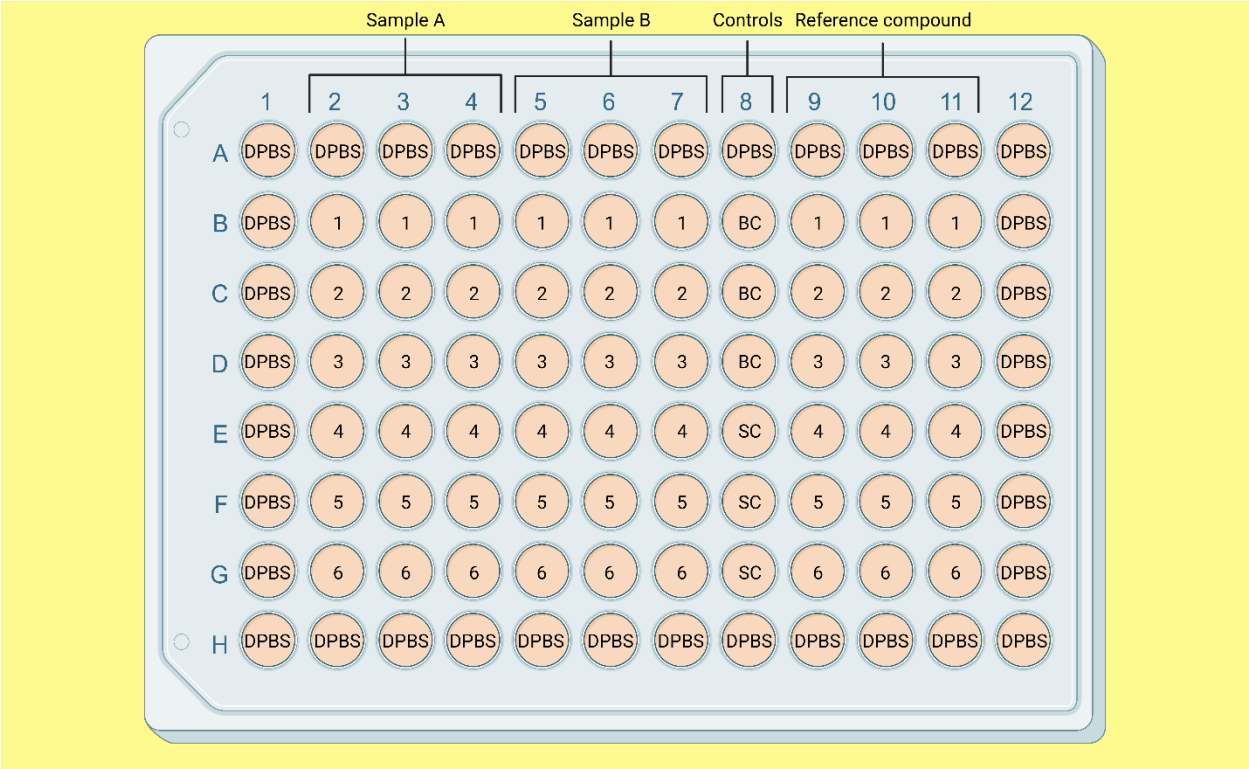

Figure 16 The 96-well plate layout for reporter gene bioassays. BC: blank control (untreated cells); DPBS: Dulbecco's phosphate buffered saline; PC: positive control; SC: solvent control; 1–6: exposure concentration 1–6.

3.7.4.1 Xenobiotic metabolism

3.7.4.1.1 H4IIE-*luc* reporter gene bioassay

Aarts *et al*. (1993) developed the H4IIE-*luc* mediated reporter gene bioassay using recombinant rat hepatoma H-4-II-E cells showing AhR-controlled luciferase expression. The AhR is a ligand-dependent transcription factor responsible for the regulation of genes involved in dioxin-like xenobiotic metabolism, cellular proliferation, and differentiation (Takeuchi *et al*., 2008), and some environmental chemical contaminants are AhR ligands (Leskinen *et al*., 2008). Consequently, the H4IIE-*luc* reporter gene bioassay was used to establish if the samples contain ligands that activate the AhR.

Aryl hydrocarbon receptor agonism

At the start of the five-day H4IIE-*luc* reporter gene bioassay (day one), the nutrient medium of the cells was stripped of hormones (DMEM supplemented with 10% CDT FBS), and the H4IIE-*luc* cells were seeded at a density of 80 000 cells/mL in 96-well, clear flat bottom, white-walled cell culture microplates (Cellstar®, Greiner Bio-One, Lasec (Pty) Ltd) and incubated for 24 hours. Following attachment after 24 hours (day two), cells were exposed to 250 µL of the samples (1, 3, 9, 28, 83 and 250 mg/mL). Positive control cells were exposed (via dosing) to the reference compound TCDD (≥ 99%) (CAS #1746-01-6, Industrial Anlytical (Pty) Ltd), a known AhR agonist and which had been serially diluted (1:5) in *n*-hexane (CAS #110-54-3, Honeywell Burdick & Jackson®, Sigma-Aldrich (Pty) Ltd) to obtain the following

concentrations: 0.06, 0.37, 2.2, 13.3, 80 and 480 pg/mL. *n*-hexane also served as the SC. On the last day of the assay (day five) and after the cells had been exposed to the samples or reference compound (TCDD) for 72 hours, the 96-well microplates were treated in the same manner as described above (Section 3.7.4) to determine luciferase activity.

3.7.4.2 Endocrine disruption

3.7.4.2.1 MDA-kb2 reported gene bioassay

The stable human breast cancer cell line MDA-kb2 was developed from MDA-MB-453 cells two decades ago by Wilson *et al*. (2002). This cell line expresses both androgen- and glucocorticoid-responsive receptors and is therefore a useful tool for studying AR and GR activity (Wilson *et al*., 2002; Aït-Aïssa *et al*., 2010). The MDA-kb2 reporter gene bioassay was thus used to determine AR and GR activity of the soil extracts.

Androgen and glucocorticoid receptor agonism

When soil extracts elicited a response during the activation assay, it could indicate AR or GR activation since both receptors are available to upregulate the MMTV promoter. On day one of the five-day MDA-kb2 bioassay for the assessment of receptor agonism, cells were seeded in 96-well, clear flat bottom, white-walled cell culture microplates (120 000 cells/mL) in L-15 medium, supplemented with 10% CDT FBS and 1% antibiotic-antimycotic mixture. Following incubation for 48 hours (day three), cells were exposed in triplicate to the soil extracts by removing the nutrient medium of the cells and replacing it with 250 µL of the samples at the following concentrations: 1, 3, 9, 28, 83 and 250 mg/mL. Since testosterone is a known AR agonist, it served as the positive control (reference compound) for AR activation. The cells were exposed to a concentration range of testosterone (≥ 99%) (8, 47, 142, 283, 567 and 850 pg/mL) (CAS #58-22-0, Sigma®, Sigma-Aldrich (Pty) Ltd, South Africa) which was diluted in methanol (CAS #67-56-1, Honeywell Burdick & Jackson®, Germany). Methanol also served as the SC. MDA-kb2 cells were exposed to the soil extracts and testosterone for 48 hours. On the last day of the MDA-kb2 assay (day five), the 96-well microplates containing the MDA-kb2 cells were treated in the same manner as described in Section 3.7.4 to determine luciferase activity.

Since both receptors are available it is important to distinguish whether compounds in the samples bind to the AR or GR. This can be done by the co-administration of a known AR antagonist, such as flutamide which specifically blocks the AR but not the GR (Wilson *et al*., 2002). Therefore, a second bioassay was performed where the AR was blocked to specifically assess GR activation. On day one, cells were seeded in the same manner as described above. However, to evaluate GR activation, the cells were seeded with a background concentration of a known AR antagonist, flutamide (0.5942 µg/mL) (≥ 99%, CAS #13311-84-7, Sigma®, Sigma-Aldrich (Pty) Ltd). Apart from exposing the cells to the dilution range of the samples (1, 3, 9, 28, 83 and 250 mg/mL) through media replacement after 48 hours of incubation, PC cells were dosed with the reference compound (known GR agonist), dexamethasone (≥ 99%,

CAS #50-02-2, Sigma®, Sigma-Aldrich (Pty) Ltd) which was serially diluted (1:3) in methanol: 0.55, 2.20, 8.79, 35.16, 140.63, and 562.50 ng/mL. Methanol also served as the SC. On the last day of the assay (day five) and after 48 hours of exposure to the soil extracts and reference compound, the 96-well microplates were treated in the same manner as described in Section 3.7.4 to determine luciferase activity. If any receptor binding of the soil extracts occurred, it was attributed to the presence of glucocorticoid receptor agonists (GR activation).

Androgen receptor antagonism

To assess the anti-androgenic activity, the MDA-kb2 cells were seeded (120 000 cells/mL) on day one of the five-day assay similarly to the AR and GR activation bioassays. However, quantifying antagonism requires the presence of a constant concentration of a known agonist (Escher *et al*., 2021) so that if an antagonist is presented, this becomes evident in a decline of light emission. Therefore, the cells were seeded with a background concentration of testosterone (0.283 ng/mL). This was done to ensure 80% activation of the AR (Escher *et al*., 2021). After 48 hours of incubation, the cells were exposed in triplicate to the dilution range of the samples (1, 3, 9, 28, 83, and 250 mg/mL), also containing testosterone (0.283 ng/mL), through media replacement to assess AR inhibition. The PC cells were also exposed to a serial dilution range (1:2) of the antagonistic reference compound, flutamide (0.02, 0.06, 0.19, 0.56, 1.67 and 5.0 µg/mL) which had been prepared in methanol. Methanol also served as the SC. The reference compound was dosed in triplicate. On the last day of the MDA-kb2 assay (day five), the 96-well microplates containing the MDA-kb2 cells were treated in the same manner as described in Section 3.7.4 to determine luciferase activity.

3.7.4.2.2 T47D-KB*luc* reporter gene bioassay

The T47D-KB*luc* human breast cancer cell line was developed by Wilson *et al*. (2004). It is a hormone-dependent cell line which responds to potent oestrogens such as E_2, EE_2 and diethylstilboestrol, as well as oestrogen-like compounds found in the environment. As a result, the T47D-KB*luc* cells can be used to screen environmental pesticide mixtures for (anti-)oestrogenic activities by evaluating binding to the ER (Wilson *et al*., 2004; Mutengwe *et al*., 2016). The T47D-KB*luc* reporter gene bioassay was therefore used to assess the agonistic and antagonistic ER activities of the soil extracts.

Oestrogen receptor agonism

Although the T47D-KB*luc* bioassay is based on the same principle as the MDA-kb2 bioassay, in the present study the ER activation bioassay could not successfully be performed according to the method prescribed in literature. This was due to the presence of an unknown source of oestrogen contamination in our tissue culture laboratory which resulted in naturally excited T47D-KB*luc* cells, influencing the outcome of the ER agonism bioassay. Consequently, several attempts were made to reduce the excitation of the cells, including (i) the use of a positively charged nylon filter to remove residual biologically active contaminants

from the ultrapure deionised water used to prepare nutrient medium for the cells; (ii) the replacement of ultrapure deionised water with high-purity water suitable for mass spectrometry for preparation of nutrient medium; (iii) the use of high-grade plastic consumables during routine cell culture maintenance; and iv) the protection of plasticware from ultraviolet (UV) light. Unfortunately, none of these changes reduced the oestrogenic contamination as further discussed in Chapters 4 and 5. In a last attempt to minimise the effect of the additional oestrogen, a background concentration of a known ER antagonist, fulvestrant (ICI), was used during the T47D-KB*luc* bioassay to ensure competitive binding for the ER between the ICI and any potential oestrogens present in the samples.

Three days prior to the T47D-KB*luc* bioassay for the assessment of ER agonism, cells were cultured in nutrient medium (RPMI-1640) which had been stripped of hormones by replacing the 10% FBS with 10% CDT FBS (Wilson *et al*., 2004). On day one of the three-day T47D-KB*luc* bioassay, cells were seeded in nutrient medium supplemented with 10% Hyclone™ CDT FBS in 96-well, clear flat bottom, white-walled cell culture microplates at a seeding density of 120 000 cells/mL. Cells were allowed to attach overnight (24 hours). On day two, the nutrient medium of the cells was removed and replaced with 250 µL of the samples (1, 3, 9, 28, 83 and 250 mg/mL) containing 0.04 ng/mL ICI. For the assessment of ER activation, PC cells were exposed via dosing to the known ER agonist, E_2 (CAS #50-28-2, Sigma®), which had been diluted in the nutrient medium supplemented with 10% Hyclone™ CDT FBS. The following E_2 exposure concentrations were used: 0.02, 0.11, 0.39, 1.36, 2.72, and 6.81 pg/mL. Nutrient medium was used to prepare the E_2 dilutions to minimise the effect of solvents on the ER. Ethanol for example has been found to stimulate ER overexpression (Chen *et al*., 2009). The nutrient media used to prepare the E_2 dilutions also contained a background of ICI (0.04 ng/mL). After 24-hour exposure to the samples or E_2 the 96-well microplates containing the T47D-KB*luc* cells were treated in the same manner as described in Section 3.7.4 to determine luciferase activity.

Oestrogen receptor antagonism

Similar to the assessment of ER agonism, cells were cultured in nutrient medium (RPMI-1640) which had been stripped of hormones by replacing the 10% FBS with 10% CDT FBS (Wilson *et al*., 2004). However, for ER antagonism the cells were cultured in the hormone-stripped medium seven days prior to the T47D-KB*luc* bioassay. This was done to ensure a better ER response during the bioassay. On day one of the three-day T47D-KB*luc* bioassay, cells were seeded in nutrient medium supplemented with 5% Hyclone™ CDT FBS in 96-well, clear flat bottom, white-walled cell culture microplates at a seeding density of 120 000 cells/mL. After 24 hours of incubation (day two), the nutrient medium of the cells was replaced with 250 µL of the samples (1, 3, 9, 28, 83 and 250 mg/mL) containing 5.4 pg/mL E_2. The exposure medium contained a background of E_2 which produced 80% of the effect (EC_{80}) on the receptor (i.e., caused 80% activation of the ER) (Escher *et al*., 2021). This is to ensure binding to and activation of the ER. In order to assess ER inhibition, the PC cells were exposed via dosing to the anti-oestrogen

reference compound, ICI (0.02, 0.05, 0.16, 0.47, 1.41 and 4.22 ng/mL) (≥ 99%, CAS #129453-61-8, European Pharmacopoeia Reference Standard, Industrial Analytical (Pty) Ltd) which had been serially diluted (1:2) in ethanol (CAS #64-17-5, LiChrosolv®, Supelco, Sigma-Aldrich (Pty) Ltd). On the last day of the T47D-KB*luc* assay (day three), the 96-well microplates containing the T47D-KB*luc* cells were treated in the same manner as described in Section 3.7.4 to determine luciferase activity.

3.7.4.3 Data calculations for reporter gene bioassays

During the reporter gene bioassays, cells were exposed to a concentration range of the reference compounds (positive controls). These concentrations were plotted on a logarithmic scale to obtain a sigmoidal concentration-response curve (Escher *et al*., 2021). Sample responses were compared to the PCs and expressed in terms of %TCDD Max (H4IIE-*luc*, AhR activation), %Testosterone Max (MDA-kb2, AR activation), %Flutamide Max (MDA-kb2, AR inhibition), %Dexamethasone Max (MDA-kb2, GR activation), $\%E_2$ Max (T47D-KB*luc*, ER activation), and %ICI Max (T47D-KB*luc*, ER inhibition). Subsequently, where possible, bioanalytical equivalents (EQs)—TCDD-EQs, testosterone-EQs, dexamethasone-EQs, flutamide-EQs, E_2-EQs, and ICI-EQs—were calculated. However, if sample responses did not resemble a classical concentration-response curve, a fold change (FC) was calculated between the maximum concentration of the positive and solvent control by dividing the mean RLUs of the highest soil extract concentration by the mean RLUs of the solvent control (Aït-Aïssa *et al*., 2010; Wilson *et al*., 2002). An FC > 1 and FC < 1 was indicative of receptor agonism and antagonism, respectively. Statistically significant ($*p \leq 0.05$) differences in RLUs between the treated (samples or reference compounds) and untreated cells were determined using SPSS. The Kolmogorov-Smirnov test was used to evaluate normality of the data but since the data was not normally distributed, the Mann-Whitney *U* test was used with a confidence interval of 95%. All data is presented as the mean response ± standard deviation (%).

3.7.5 Enzymatic and non-enzymatic bioassays

3.7.5.1 Oxidative stress responses

3.7.5.1.1 Harvesting of cell content

For the determination of different oxidative stress endpoints, the cell contents of the HuTu-80 and H4IIE-*luc* cells had to be harvested. At the start of all the oxidative stress bioassays (day one), HuTu-80 and H4IIE-*luc* cells were seeded at a density of 80 000 cells/mL in 24-well, clear, flat bottom cell culture microplates (Corning® Costar®, The Scientific Group (Pty) Ltd) in DMEM (**Figure 17**; **Figure 18**; **Table 1**). Cells were incubated for 24 hours. Following attachment (day two), cells were exposed in triplicate to the soil extracts by removing the nutrient medium of the cells and replacing it with 1 000 µL of the samples (83 mg/mL). Untreated cells were also included to act as a control. After 24 hours of exposure, the media of all the cells were removed and the cells were gently washed three times with 500 µL

DPBS and detached from the 24-well microplate by the addition of 150 µL of trypsin for 3 minutes. The trypsin activity was stopped by the addition of 500 µL DPBS. The cell suspension was centrifuged for 4 minutes at 1 000 *g* and 25°C in 2 mL microcentrifuge tubes (Axygen, Lasec (Pty) Ltd). The supernatant was discarded, and the cell pellet re-suspended in 270 µL ice-cold potassium phosphate buffer consisting of 0.09 M dipotassium hydrogen phosphate (K_2HPO_4) (CAS #7758-11-4, Sigma®, Sigma-Aldrich (Pty) Ltd) adjusted to pH 7.4 with 0.09 M potassium dihydrogen phosphate (KH_2PO_4) (CAS #7778-77-0, Sigma®, Sigma-Aldrich (Pty) Ltd). Subsequently, the cell suspension was lysed through ultrasonication at medium intensity for 30 seconds and centrifugation at 10 000 *g* for 4 minutes (4°C). This cell lysate supernatant (supernatant A) was used for the determination of protein and SOD content, as well as CAT activity.

For measurement of the MDA content (LPO) and AChE activity, the same harvesting procedure was followed to obtain the cell lysate supernatant (supernatant B), but the resulting cell pellet was re-suspended in 200 µL tris-hydrochloric acid (HCl)/sucrose buffer and not the phosphate buffer as describe for protein and SOD content, and CAT activity. The tris-HCl/sucrose buffer was prepared by adding tris-HCl (25 mM) (CAS #1185 53 1, Trizma® hydrochloride, Sigma-Aldrich (Pty) Ltd) to sucrose (250 nM) (CAS #57-50-1, Sigma®, Sigma-Aldrich (Pty) Ltd) in a 1:1 (*v*/*v*) ratio. The samples were vortexed vigorously and sonicated at medium intensity for 30 seconds to lyse the cells, followed by centrifugation for 10 minutes at 4°C and 10 000 *g*.

3.7.5.1.2 Intracellular reactive oxygen species production

Reactive oxygen species are highly reactive chemicals formed from O_2 and their production is part of normal metabolic processes in aerobic organisms. When present in physiological concentrations, ROS play a key role in cellular homeostasis and maintains biological functions, such as regulation of gene expression, cell proliferation, survival, apoptosis, tissue development and maintaining organ function (Pisoschi & Pop, 2015; Ventura *et al*., 2015; Jabłońska-Trypuć *et al*., 2017). However, higher cellular concentrations lead to oxidative stress and cell damage (Chang *et al*., 2020). The level of ROS production in cells can be directly measured using the fluorogenic dye H_2DCF-DA. The fluorogenic dye diffuses into the cells and is hydrolysed to non-fluorescent DCFH by intracellular esterases where it remains trapped within the cells. If H_2O_2 is produced by the cells as a result of increased oxidative stress, the DCFH will react with the H_2O_2 and generate the highly fluorescent DCF, which can be quantified using a microplate reader (Wang & Joseph, 1999; Katerji *et al*., 2019).

Reactive oxygen species generation due to exposure to the soil extracts was determined according to the methods described by Wang and Joseph (1999), and Halliwell and Whitemann (2004), with a few modifications. At the start of the assay (day one), HuTu-80 and H4IIE-*luc* cells were seeded in DMEM (**Table 1**) in 24-well microplates as shown in **Figure 17** (ROS) and **Figure 18** (protein and SOD content,

CAT activity, LPO and AChE activity), and incubated as previously described. Following attachment after 24 hours, cells were exposed in triplicate to the samples by replacing the nutrient medium with 1 000 µL of the prepared soil exposure medium (83 mg/mL). After another 24-hour incubation period, the positive control cells were incubated with 3.5 ng/mL H_2O_2 (CAS #7722-84-1, Sigma®, Sigma-Aldrich (Pty) Ltd) for 45 minutes. After stimulation with H_2O_2, the media of all the cells (treated, untreated, and positive control cells) were removed and the cells were gently washed three times with 500 µL DPBS. From here on the rest of the assay was performed in the dark since the fluorogenic dye probe is light sensitive. 200 µL H_2DCF-DA (10 µM) (CAS #4091-99-0, Sigma®, Sigma-Aldrich (Pty) Ltd) was added and the cells were incubated at 37°C (5% CO_2) in the dark for 30 minutes. After the H_2DCF-DA was removed, the cells were washed three times with 500 µL DPBS and trypsinised. Trypsin activity was stopped by the addition of 500 µL DPBS. The cell suspension was transferred into a 2 mL microcentrifuge tube and centrifuged for 4 minutes at 1 000 *g* and 25°C. Subsequently, the supernatant was discarded, and the cell pellet re-suspended in 800 µL DPBS. To quantify fluorescence, 200 µL of the cell suspension was transferred to a 96-well, black, flat bottom cell culture microplate (Corning®, Sigma-Aldrich (Pty) Ltd, South Africa). The cell contents of one 24-well plate were shared between three wells in the 96-well plate, realising nine fluorescent readings for one sample. Fluorescence was measured as relative fluorescence units (RFUs) at excitation and emission wavelengths of 480 nm and 535 nm, respectively, using a SpectraMax® iD3 multi-mode microplate reader (Molecular Devices, LCC, Lasec SA (Pty) Ltd, South Africa). Since the emitted DCF fluorescence is directly proportional to the amount of ROS produced intracellularly (Mattia *et al*., 1991), the ROS produced by the cells after exposure were calculated based on the difference between the RFUs of treated and untreated cells (blank control) (Yao *et al*., 2015).

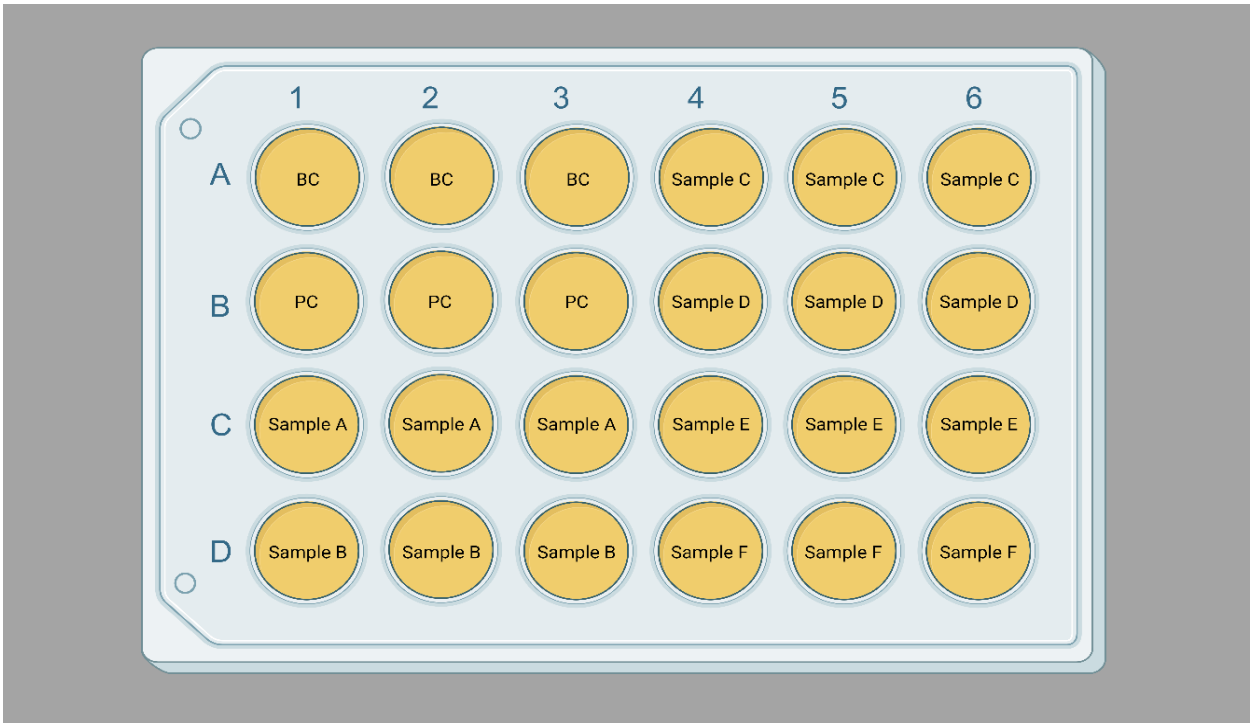

Figure 17 The 24-well plate layout for the determination of reactive oxygen species production. BC: blank control (untreated cells); PC: positive control (hydrogen peroxide stimulated cells) (Created in Biorender.com).

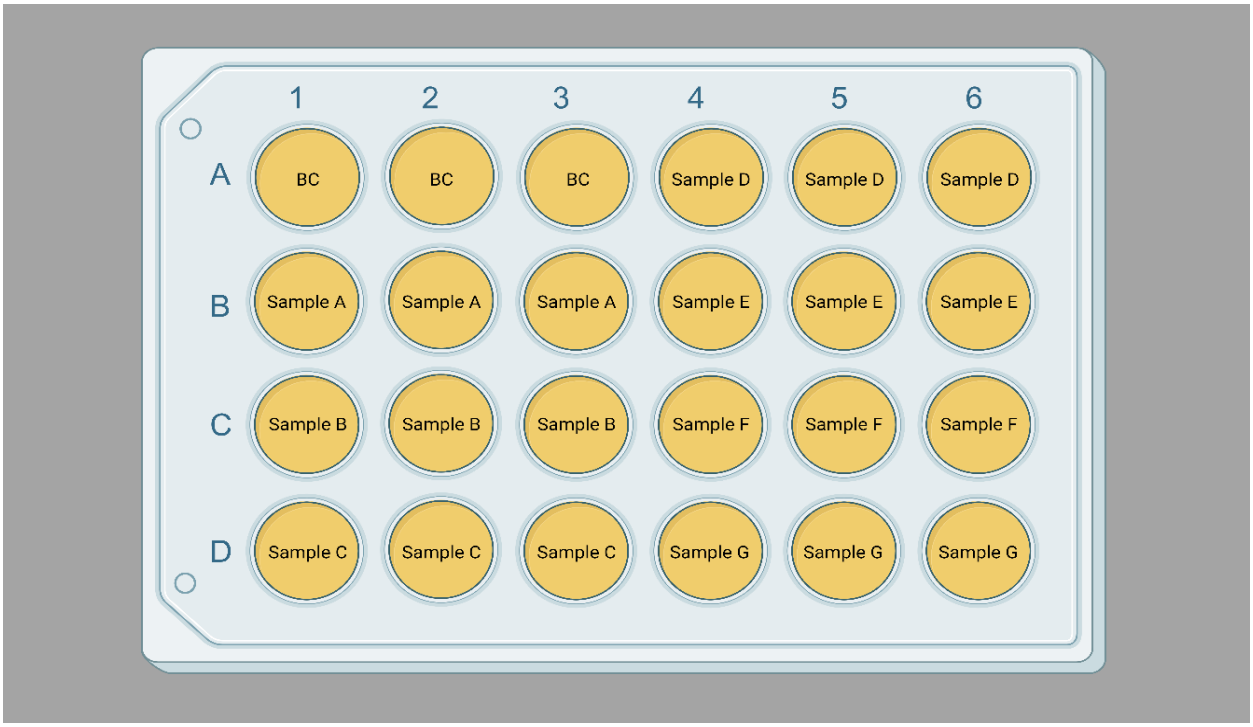

Figure 18 The 24-well plate layout for the determination of protein and superoxide dismutase content, catalase activity, lipid peroxidation and acetylcholinesterase activity. BC: blank control (untreated cells) (created in BioRender.com).

3.7.5.1.3 Protein content

Total protein content represents the number of cells per well during an assay performed in a cell culture microplate. As a result, the protein content in HuTu-80 and H4IIE-*luc* cells exposed to the samples was determined as a measure to normalise all antioxidant enzyme activity data. Specific SOD content and CAT activity were expressed in terms of units per milligram protein (ng/mg protein and µM H_2O_2/min/mg protein for SOD and CAT, respectively) (Santovito *et al*., 2005). The total protein concentration was determined according to the method described by Bradford (1976) using bovine serum albumin (BSA) as standard. The Bradford protein assay is a colourimetric assay based on the principle that under acidic conditions the red, cationic form of Coomassie brilliant blue G-250 dye will be converted into its blue, anionic form when it binds to proteins. This causes a shift in the absorption maximum of the dye from 465 nm to 595 nm and the increase in absorption can therefore be monitored at 595 nm. However, if no proteins are present to bind to the dye, the solution will remain brown (Bradford, 1976).

For the determination of total protein concentration, 5 µL of sample supernatant A (Section 3.7.5.1.1) was transferred to a 96-well, white-walled, flat bottom cell culture microplate in triplicate, followed by the addition of 245 µL of Bradford's reagent (Supelco, Sigma-Aldrich (Pty) Ltd)—an acidified solution of Coomassie Brilliant Blue G-250—in the dark. Protein content was quantified by measuring absorbance at 590 nm using a Berthold multimode microplate reader. Measurements were performed in triplicate. A protein standard calibration curve was prepared using BSA (CAS #9048-46-8, Sigma-Aldrich (Pty) Ltd) which was serially diluted (1:1) (0–2 000 µg/mL), serving as the positive control. The BSA protein concentration (µg/mL) (x-axis) was plotted against the corresponding absorbance value (y-axis) to obtain a protein calibration curve with the following linear equation:

$$y = mx + c$$

with

y = relative response (absorbance)
m = the gradient of the straight line
x = protein concentration (µg/mL)
c = the y-intercept of the straight line

This standard curve uses linear regression to calculate the protein concentration (µg/mL) within cells exposed to samples (soil extracts) (Bradford, 1976). The total protein concentration of cells after exposure to samples was converted from µg/mL to mg/mL and used in the data calculations for SOD content (Section 3.7.5.1.4), CAT activity (Section 3.7.5.1.5), LPO as MDA content (Section 3.7.5.1.6) and AChE activity (Section 3.7.5.2).

3.7.5.1.4 Superoxide dismutase content

Superoxide dismutase scavenges univalent reduced oxygen by catalysing the partitioning of these $O_2^{\bullet-}$ into H_2O_2 and O_2. The removal of $O_2^{\bullet-}$ by SOD activity can be determined by measuring the reduction in the rate of autoxidation of organic compounds, such as pyrogallol (2,3-dihydroxyphenol), which rapidly occurs in the presence of $O_2^{\bullet-}$. The pyrogallol assay is based on the principle that SOD within cells will convert the $O_2^{\bullet-}$ into ordinary O_2 and H_2O_2, and as a result, white pyrogallol will not autoxidize into a variety of yellow-brown oxidation products. A yellow-brown colour indicates autoxidation and no SOD activity, while a white colour indicates no autoxidation and SOD activity (Marklund & Marklund, 1974). Superoxide dismutase activity can therefore be quantified by recording this kinetic reaction.

The pyrogallol autoxidation method described by Marklund and Marklund (1974), and Del Maestro and McDonald (1987) were used to determine SOD content. A 4 µL aliquot of sample supernatant A prepared in Section 3.7.5.1.1 was added in triplicate to a 96-well, white-walled, clear flat bottom cell culture microplate. 245 µL of a 1:49 (*v*/*v*) solution containing 1 nM diethylenetriaminepentaacetic acid (DTPA) (CAS #67-43-6, Sigma®, Sigma-Aldrich (Pty) Ltd, South Africa) and 50 mM tris buffer [tris(hydroxymethyl)aminomethane)] (CAS #77-86-1, Sigma-Aldrich (Pty) Ltd) (pH 7.5) was added to each well. During preparation, the DTPA/tris buffer was aerated for 20 minutes, and the pH was adjusted to 8.2 with HCl (32%) (CAS #7647-01-0, Sigma-Aldrich (Pty) Ltd). From here on the rest of the assay was performed in the dark since pyrogallol is light sensitive. The addition of 4 µL of pyrogallol (24 nM in 10 mM HCl-acidified deionised water) (CAS #87-66-1, Sigma®, Sigma-Aldrich (Pty) Ltd) initiated the reaction. The kinetic reaction was recorded by measuring optical density at 560 nm every 30 seconds for 4.5 minutes starting at time zero using a Berthold multimode microplate reader (10 time points in total). Measurements were performed in triplicate. For data calculation, the mean absorbance and reaction gradient were determined. The reaction rate (change in absorbance over 4.5 minutes) was calculated and normalised to percentage. One unit of SOD is defined as the amount of enzyme necessary to inhibit 50% of the reaction (pyrogallol autoxidation). Consequently, the %inhibition was divided by 50 to determine the SOD content in units. The amount of SOD in units was multiplied by 125 and expressed as ng SOD (one SOD unit equals 125 ng SOD). To obtain the fraction responsible for SOD content in µL, the amount of ng SOD was multiplied by the dilution factor (i.e., 4). This was followed by converting ng SOD/µL to ng SOD/mL. Lastly, obtained values were normalised against protein content in each cell lysate (Section 3.7.5.1.3) and expressed as SOD content in ng SOD/mg protein (Marklund & Marklund, 1974; Del Maestro & McDonald, 1987).

3.7.5.1.5 Catalase activity

Catalase is the enzyme responsible for converting reactive H_2O_2 into H_2O and O_2 (Chance, 1948). The determination of CAT activity is therefore based on the principle of measuring the enzyme-catalysed decomposition of H_2O_2. The amount of H_2O_2 remaining after catalase action in the cells was determined by

titration with an excess of a very strong oxidizing reagent, $KMnO_4$. The resulting colour response is quantified spectrophotometrically and the amount of residual $KMnO_4$ is inversely proportional to the activity of the catalase enzyme. The CAT assay was performed according to the method described by Cohen *et al*. (1970) as follows: 4 µL sample supernatant A prepared in Section 3.5.7.1.1 was added in triplicate to a 96-well, white-walled, flat bottom cell culture microplate. The reaction was initiated by the addition of 93 µL H_2O_2 (6 mM), followed by incubation at 37°C for 3 minutes. After incubation the reaction was stopped by the addition of 19 µL of H_2SO_4 (6 M) (CAS #7664-93-9, Sigma®, Sigma-Aldrich (Pty) Ltd). From here on the assay was performed in the dark. To quantify catalase activity, the amount of H_2O_2 remaining after catalase action was determined by adding 130 µL $KMnO_4$ (1.9 mM) (CAS #7722-64-7, Sigma®, Sigma-Aldrich (Pty) Ltd, South Africa) to the cells. The $KMnO_4$ which did not react (residual) with the H_2O_2 is determined spectrophotometrically by immediately (within 30 to 60 seconds) measuring absorbance at 490 nm using a Berthold multimode microplate reader. Measurements were performed in triplicate. A CAT blank comprised of only reagents [$KMnO_4$, potassium phosphate buffer and H_2SO_4] and no cells was also included in triplicate (experimental blank). Under the conditions described the enzyme-catalysed decomposition of H_2O_2 by CAT follows first-order kinetics as given by the following equation:

$$k = \log(S_0/S_3) \times 2.3t$$

with

k = the first-order reaction rate constant
t = the time interval over which the reaction is measured (3 minutes)
S_0 = the substrate concentration at time zero
S_3 = the substrate concentration at time 3 minutes
2.3 = the first-order kinetic conversion factor

S_0 was the mean absorbance of the CAT standard. To obtain S_3, the individual absorbance values of the reaction samples were subtracted from the S_0. After the calculation of the first-order reaction rate constant, k, obtained values were normalised against protein content in each cell lysate (Section 3.7.5.1.3). The values were converted to µM by multiplying with 1 000 and consequently expressed as µM H_2O_2/min/mg protein (Cohen *et al*., 1970; Mennillo *et al*., 2019).

3.7.5.1.6 Lipid peroxidation as malondialdehyde content

Lipid peroxidation occurs when free radicals bind to a carbon on the alkyl chain of a fatty acid, leading to the oxidative degradation of lipid components within the cellular membranes of an organism. During this process, reactive aldehydes such as MDA and 4-hydroxynonenal are formed as by products and can be used

to measure LPO. Lipid peroxidation was estimated through quantitation of MDA levels using the TBARS assay according to Ohkawa *et al*. (1979) as modified by Boussabbeh *et al*., (2016) for tissue cultures. The TBARS assay is a colourimetric assay that involves the reaction of the LPO degradation product, MDA, with TBA under conditions of high temperature and acidity to generate a pink coloured adduct which can be quantified spectrophotometrically (Almroth *et al*., 2005).

For determining MDA content, a calibration curve was also prepared using 1,1,3,3-tetramethoxypropane (TMP) (CAS #102-52-3, Sigma®, Sigma-Aldrich (Pty) Ltd, South Africa) as the MDA precursor and which was diluted with deionised water, resulting in the following concentrations: 60, 90, 120, 150, 180, 210, and 240 mM. A 12.5 µL aliquot of the sample supernatant B (Section 3.7.5.1.1), MDA standards and tris-HCl/sucrose buffer serving as the experimental blank was transferred to separate 15 mL centrifuge tubes. The following reagents were added to each centrifuge tube: 25 µL sodium dodecyl sulphate (SDS; 8.1%) (CAS #151-21-3, Sigma®, Sigma-Aldrich (Pty) Ltd) solution; 187.5 µL glacial acetic acid ($CH_3COOH_{(c)}$, 20%, pH 3.5) (CAS #64-19-7, Merck Chemicals (Pty) Ltd); 187.5 µL TBA (0.8%) (CAS #504-17-6, Sigma®, Sigma-Aldrich (Pty) Ltd); and 87.5 µL deionised water. After the addition of reagents, the centrifuge tubes were incubated for 30 minutes at 95°C in a water bath. Once cooled to room temperature, 125 µL deionised water and 625 µL of *n*-butanol (CAS #71-36-3, Honeywell Riedel-de-Haën™, Germany) and pyridine (CAS #110-86-1, Sigma®, Sigma-Aldrich (Pty) Ltd) solution (15:1, *v*/*v*) were added. The samples, MDA standards and experimental blanks were vortexed and centrifuged for 10 minutes at 2 700 *g* (25°C) to allow separation of the aqueous and organic phases. A 245 µL aliquot of the organic phase (upper pink-coloured layer) was added in triplicate to a 96-well, white-walled, clear flat bottom cell culture microplate. Malondialdehyde content was quantified by measuring absorbance at 532 nm using a SpectraMax® iD3 multi-mode microplate reader. The TMP concentration (mM) (x-axis) was plotted against the corresponding absorbance value (y-axis) to obtain an MDA calibration curve with the following linear equation:

$$y = mx + c$$

with

y = relative response (absorbance)
m = the gradient of the straight line
x = TMP concentration (mM)
c = the y-intercept of the straight line

This standard curve uses linear regression to calculate the MDA concentration (mM) within cells exposed to samples (soil extracts). Obtained values were normalised against protein content (Section 3.7.5.1.3) and expressed as mM MDA/mg protein.

3.7.5.2 Acetylcholinesterase inhibition

Non-neuronal AChE is produced in many cells throughout the body, including in the liver and intestines. Acetylcholinesterase controls the amount of AChE by hydrolysing the ACh to choline and acetate upon completion of signal transmission (Quinn, 1987). Acetylcholinesterase plays an important role in gene expression, cell proliferation, and differentiation (Wessler *et al*., 2001; García-Ayllón *et al*., 2006). Consequently, AChE inhibition can indicate damage to non-neuronal cholinergic transmission in important detoxification organs (i.e., intestines and liver).

Acetylcholinesterase activity was determined according to Ellman *et al*. (1961). However, the Ellman method which used cuvettes was adapted for measurement in a 96-well microplate (Zimmerman *et al*., 2008; Härtl *et al*., 2011). The Ellman assay is a colourimetric assay based on measuring the colour response when thiocholine reacts with Ellman's reagent (DTNB) producing yellow TNB. The colour response is quantified spectrophotometrically and the measured absorbance of TNB is proportional to AChE activity. For the determination of AChE activity, the following reagents were added to a 96-well, white-walled, clear flat bottom cell culture microplate: 210 µL potassium phosphate buffer; 10 µL acetylthiocholine iodide (30 mM) (CAS #1866-15-5, Sigma®, Sigma-Aldrich (Pty) Ltd, South Africa) and 10 µL Ellman's reagent (10 mM; prepared in methanol) (CAS #69-78-3, Sigma®, Sigma-Aldrich (Pty) Ltd, South Africa). The reagents were gently mixed by tapping the cell culture microplate, followed by incubation at 37°C for 5 minutes. Subsequently, 10 µL of sample supernatant B prepared in Section 3.7.5.1.1 was added in triplicate to the 96-well microplate. Experimental blanks containing only potassium phosphate buffer, acetylthiocholine iodide and DTNB were also included. Absorbance was immediately measured at 412 nm every minute for a period of 6 minutes starting at time zero (7 time points in total) using a SpectraMax® iD3 multi-mode microplate reader. To determine AChE activity, the mean absorbance of each time interval was determined for the samples, untreated cells, and experimental blanks. This was followed by calculating the reaction gradient. The reaction rate (change in absorbance over 6 minutes) was determined, after which the obtained values were normalised against protein content in each cell lysate (Section 3.7.5.1.3) and expressed as AChE activity in absorbance/min/mg protein (Ellman *et al*., 1961).

3.7.5.3 Statistical analysis of data for enzymatic and non-enzymatic bioassays

Statistical analysis of data for oxidative stress responses (ROS, SOD content, CAT activity and MDA content) and AChE activity was performed using Microsoft Excel and the SPSS statistical package version 24 (IBM) to determine statistically significant differences in responses between treated (exposed) and untreated cells (blank control). The Kolmogorov-Smirnov test was used to test for normality within the

data. Since the data was not normally distributed, the Mann-Whitney *U* test was used with a statistical significance threshold of 0.05 and 0.01. Cohen's d-value was also determined for all responses to establish the effect size (i.e., practical significance):

$$d = \frac{|\bar{x}_E - \bar{x}_K|}{S_K}$$

with

d = Effect size (i.e., practical significance)

$\bar{x}_E$ = Mean of the sample group

$\bar{x}_K$ = Mean of the control group

S_K = Standard deviation of the control group

When d = 0.8 the difference between the sample and control group has a large effect. Consequently, an effect size of $d \geq 0.8$ was considered practically significant (Ellis & Steyn, 2003). Spearman's rho coefficient (r_s) was determined to investigate the association between the %TOC of the soil from the respective sampling locations and the oxidative stress responses (ROS, SOD, and CAT) observed for the HuTu-80 and H4IIE-*luc* cell lines. Since bioanalytical EQs could not be calculated for AhR, AR, and ER activity, correlation analysis was only performed for the %TOC and oxidative stress responses. Spearman's rho coefficient (r_s) values range between -1 and +1 (Akoglu, 2018), with absolute values ($|r_s|$) used to interpret the strength of the linear relationship: $|r_s| = 0$ (no correlation); $|r_s| \leq 0.3$ (poor correlation); $0.3 < |r_s| \leq 0.5$ (fair correlation); $0.6 \leq |r_s| \leq 0.8$ (moderately strong correlation); $|r_s| > 0.8$ (very strong correlation) (Chan, 2003). Moreover, the direction of the correlation is indicated by the sign of r_s: positive (+) or negative (-) correlation (Akoglu, 2018). All data for the enzymatic and non-enzymatic bioassays are presented as the mean ± standard deviation.

3.8 Instrumental chemical analysis

Complementary to the *in vitro* bioassays (Section 3.7), a UHPLC-QTOF/MS was used to determine the concentration of four pesticides (quantification) and the presence of other pesticides (screening) in the polar (water-soluble) fraction of the soil samples. The four pesticides—2,4-D, atrazine, dicamba, and imidacloprid—were selected due to their use in the agriculture sector of South Africa and expected concentration in the soil. The approach followed in this study was specifically for the separation, detection and quantification of polar compounds present in the soil extracts.

3.8.1 Extraction of polar compounds from soil samples

The soil samples were air-dried, ground into a fine powder with mortar and pestle, and passed through a 600 µM steel mesh sieve (200 mm in diameter) (Clear Edge Test Sieves, South Africa) to create a homogenous sample matrix. The maize and pecan soil samples were extracted using accelerated solvent extraction (ASE). This method is based on the extraction of compounds from a solid sample (e.g., soil) under elevated temperature and pressure, over a short period of time and by using small volumes of a solvent (Richter *et al*., 1996). Accelerated solvent extraction has been successfully applied in the extraction of pesticide residues in soil and was thus considered suitable for the present study (Gan *et al*., 1999; Guzzella & Pozzoni, 1999; Riedo *et al*., 2021).

The target compounds were extracted from 80 g soil, which had been spiked with the internal standard caffeine (CAS #58-08-2; 0.005 µg/mL) prior to extraction (Meng, 2008), using the DIONEX ASE 100® instrument (Thermo Scientific, California, USA) with 100 mL stainless steel extraction cells. All the glassware and ASE extraction cells were pre-washed with deionised water (18.2 MΩ-cm) and rinsed with methanol. Accelerated solvent extraction was performed according to the method described by Vonberg *et al*. (2014) with a few modifications. Methanol was used as the extraction solvent and the parameters for the DIONEX ASE 100® were set to 60˚C, 10 342 kPa, 10 minutes heat with 5 minutes static, 60% flush volume, nitrogen purge time of 100 seconds, and three cycles. The crude extract was collected in a collection bottle. A gentle stream of nitrogen gas was used to evaporate the extract until dryness using a TurboVap® II concentration workstation (Caliper Life Sciences Inc, Massachusetts, USA). The samples were reconstituted with 1 mL methanol containing 1% formic acid (CAS #64-18-6, Honeywell Fluka™). The extracts were passed through a 0.22 µm Acrodisc® prefilter to remove any particulates before being subject to chemical analysis.

3.8.2 Quality control and quality assurance

Quality control was performed to evaluate the quality of the extraction method and chemical analysis. The method was validated for the following parameters: matrix effects, linearity, precision, and accuracy. The LOD and LOQ were also determined. Matrix effects can influence the results obtained during liquid chromatography-mass spectrometry analysis. Therefore, an external matrix-matched calibration curve (spiked post-extraction) was used to account for matrix effects (Wu *et al*., 2022). A matrix-matched-calibration curve was prepared by extracting 80 g of blank soil and spiking it with pesticide standards and caffeine (internal standard) post-extraction. The pesticide standard concentrations were prepared to deliver a linear range of the target compounds as listed in **Table 2**. Coefficients of determination (R^2) (as close to one as possible) was used for the assessment of the linearity of the matrix-matched calibration curve (Miller & Miller, 2010). Quality control (QC) samples were spiked with low, medium, and high concentrations of the target pesticides (**Table 3**) to determine the recovery (%) of the ASE extraction method. The accuracy was presented as the percentage of target compounds recovered from the QC sample in three concentrations

(low, medium, and high). The low, medium, and high concentrations of the target compounds were selected based on the matrix-matched-calibration curve. Precision was expressed as relative standard deviation (RSD). Intra-day and inter-day precision were also evaluated (Wu *et al*., 2022). For the former, this was done by injecting six replicates of a QC sample from the same day, while the latter was based on a QC sample injected six times a day, over three days. The LOD and LOQ were calculated as follow (Schoeman *et al*., 2015):

$$LOD = 3*Sa/b$$
$$LOQ = 10*Sa/b$$

with

Sa = Standard deviation of the intercept (abundance)
b = slope of the external matrix-matched calibration curve

The concentrations of the target compounds (i.e., pesticide standards) were calculated based on matrix-matched calibrations run at known concentrations (x) to obtain their corresponding peak area (abundance) responses (y). The sample extracts of unknown concentrations were analysed in triplicate and based on the following linear regression formula their relative responses (y) were used to calculate their concentrations (x):

$$y = mx + c$$

with

y = relative response (abundance)
m = slope
x = concentration (µg/mL)
c = y-intercept

A blank was also included and extracted, evaporated, and concentrated in the same manner as the samples to demonstrate that there was no contribution of target compounds during the sample processing steps. Detected pesticide concentrations (µg/mL) were converted to µg/g dry soil and expressed as the mean ± standard deviation.

Table 2 Pesticide concentrations (µg/mL) of target compounds used for the matrix-matched calibration curve.

Nr	2,4-D	Atrazine	Dicamba	Imidacloprid
1	0.000	0.000	0.000	0.000
2	0.050	0.005	0.750	0.500
3	0.850	0.008	1.200	1.200
4	0.250	0.013	2.000	2.500
5	0.500	0.030	3.800	4.000
6	0.800	0.050	6.000	6.000
7	1.300	0.075	10.500	8.000
8	2.000	0.100	15.000	10.000

2,4-D: 2,4-Dichlorophenoxyacetic acid

Table 3 Concentrations (µg/mL) of target compounds in the quality control samples.

Concentration	2,4-D	Atrazine	Dicamba	Imidacloprid
Low	0.085	0.008	1.200	1.200
Medium	0.500	0.030	3.800	4.000
High	1.300	0.075	10.500	8.000

2,4-D: 2,4-Dichlorophenoxyacetic acid

3.8.3 Quantification of 2,4-D, atrazine, dicamba, and imidacloprid, and data calculations

Chemical analysis of the soil samples was performed according to a method adapted from Meng (2008). A UHPLC-QTOF/MS system comprised of an Agilent 1290 Infinity binary pump (#G4220A), autosampler (#G4226A) and thermostatted column compartment (#G1316C, Agilent Technologies, Inc. California, United States of America); coupled to an Agilent 6540 Accurate-Mass Q-TOF/MS (#G6540A, Agilent Technologies, Inc. California, United States of America) analysis was performed by injecting 1 µL of the soil extract into an Agilent ZORBAX Eclipse Plus C18 Rapid Resolution HD (2.1 mm x 50 mm, 1.8 µm particle size) column (#959757-902, Agilent Technologies, Inc. California, United States of America) for the separation of peaks. The column temperature was maintained at 60°C. The mobile phases consisted of (A) ultrapure water containing 0.1% formic acid (*v*/*v*) and (B) acetonitrile (CAS #75-05-8, Honeywell Burdick & Jackson®, Germany) containing 0.1% formic acid (*v*/*v*) with a binary pump flow rate of 0.3 mL/minute. To ensure optimum chromatographic separation the following gradients were used for positive (**Table 4**) and negative (**Table 5**) modes:

The ionisation of compounds present in the soil extracts was achieved by positive and negative ESI that were enhanced with Agilent Jet Stream technology. The mass axis of the Q-TOF was calibrated for both ionisation states daily using the Agilent tuning mix (#G1969-85000, Agilent Technologies, Inc. California, United States of America). For accurate mass references, a solution containing masses 121.050873 $[M+H]^+$

and 922.009798 $[M+H]^+$ was constantly infused. The drying gas temperature was set to 200°C, with a gas flow of 9 L/min and nebuliser pressure of 276 kPa. Sheath gas temperature and gas flow were 250°C and 10 L/min, respectively. The following voltage specifications were used: capillary voltage of 3 500 V; nozzle voltage of 1 000 V; fragmentor voltage of 175 V; skimmer voltage of 35 V; and octopole RF peak-to-peak voltage of 750 V. The instrument was set to scan from 100 to 3 000 m/z and was operated in the extended dynamic range mode (2 GHz) at 1 spectra/second. The total run-time was 22 and 10 minutes per run for positive and negative mode, respectively. The following analytical grade pesticide standards were used: 2,4-D (CAS #94-75-7); atrazine (CAS #1912-24-9); dicamba (CAS #1918-00-9); and imidacloprid (CAS #138261-41-3). As previously mentioned, caffeine was used as the internal standard (Meng, 2008). All the standards were obtained from Sigma-Aldrich (Pty) Ltd. Detection was based on the specific retention time and m/z of the target compounds (**Table 6**).

Table 4 Gradient used for the mobile phase during the positive electrospray ionisation method.

Gradient (min)	Mobile phase A (%) Ultrapure water + 0.1% formic acid	Mobile phase B (%) Acetonitrile + 0.1% formic acid
4.00	85.00	15.00
4.10	75.00	25.00
12.00	75.00	25.00
12.10	55.00	45.00
15.00	55.00	45.00
15.10	0.00	100.00
20.00	0.00	100.00
20.10	85.00	15.00
22.00	85.00	15.00

Table 5 Gradient used for the mobile phase during the negative electrospray ionisation method.

Gradient (min)	Mobile phase A (%) Ultrapure water + 0.1% formic acid	Mobile phase B (%) Acetonitrile + 0.1% formic acid
2.00	95.00	5.00
2.10	70.00	30.00
6.00	70.00	30.00
6.10	0.00	100.00
7.00	0.00	100.00
7.10	95.00	5.00

Table 6 Mass-to-charge (m/z) ratios of precursor and product ions, and retention times for target compounds.

Compound	Precursor ion (m/z)	Product ion (m/z)	Retention time (min)	Ion Polarity
2,4-D product	218.9631	160.9577	6.00	Positive
Atrazine	216.1057	216.1057	8.80	Positive
Caffeine	195.0901	195.0901	1.00	Positive
Dicamba product	218.9636	174.9737	4.50	Negative
Imidacloprid	256.0622	256.0622	2.50	Positive

2,4-D: 2,4-Dichlorophenoxyacetic acid.

The data obtained after the chromatographic analysis of the soil extracts by an UHPLC coupled to a Q-TOF/MS was used to quantify the target compounds (i.e., 2,4-D, atrazine, dicamba, and imidacloprid). The software used for data processing was Agilent's MassHunter Data Acquisition (Version 10.1 B.10.1.733.0) and Quantitative Analysis for Q-TOF (Version 10.0 B.10.0.10305.0).

3.8.4 Screening for the presence of other agrochemicals

The data obtained after the chromatographic analysis of soil extracts by UHPLC-QTOF/MS was also used to screen for other polar pesticides present in the soil extracts. Data extraction was performed using Agilent's MassHunter Quantitative Analysis for Q-TOF. The Find-by-Molecular-Feature extraction algorithm was used according to Agilent's specifications to aid with data extraction. Subsequently, compound possibilities were generated based on molecular features and the spectra were identified by spectral analysis using Agilent's MassHunter Forensics and Toxicology (Version B.04.00; #G3876CA, Agilent Technologies, Inc. California, United States of America) Personal Compound Database and Library (PCDL). This PCDL uses accurate monoisotopic mass, isotope patterns, fragment confirmations and retention times of compounds to confirm their presence. The Forensics and Toxicology PCDL contains a curated accurate-mass database with over 9 200 compounds representing various compound classes of forensic toxicology, some which include agrochemicals (acaricides, fungicides, herbicides, insecticides, and plant growth regulators). After matching the chromatographic data obtained to the Forensics and Toxicology PCDL, the data was exported to Microsoft Excel® for further processing. Only compounds with an abundance and score above 1 000 and 50, respectively, were further investigated. All the compounds that were detected in the blank with abundance > 10 000 were excluded from the results obtained for the samples.

CHAPTER 4: RESULTS

In this study, the biological effects (endocrine disruption, oxidative stress and damage, and acetylcholinesterase inhibition) associated with the water-soluble (bioavailable) fraction of maize and pecan soil were assessed. This was done by extracting polar compounds from the soil using pH-adjusted deionised water to simulate environmental conditions: the movement of applied agrochemicals from the point of application towards non-target locations following rainfall and irrigation. This chapter describes the results obtained for the soil properties investigated, various *in vitro* bioassays performed, and instrumental chemicals analysis.

4.1 Soil properties

Soil properties govern the behaviour of agrochemicals in the environment and influence how these chemicals bind to soil particles. This directly influences the binding affinity, partitioning and bioavailability of these chemicals. Consequently, total organic carbon content (%TOC) and soil particle size distribution (% composition) were assessed to determine how easily the polar compounds present in the soil could move into aquatic environments and elicit biological effects on non-target biota.

4.1.1 Total organic carbon content

Total organic carbon refers to the total amount of oxidisable carbon (dissolved, particulate, and suspended organic matter) in soil. The TOC plays a role in the adsorption of chemicals to carbon molecules in the soil. The %TOC was determined using the semi-quantitative method of loss-on-ignition. Based on the results obtained the soil was classified as having a very low (< 0.05%); low (0.05–1%); moderately low (1–2%); medium (2–4%); or high (> 4%) carbon content (Gerber *et al*., 2015). **Table 7** lists the TOC content (%) for the maize field and pecan orchard soil sampled in MP and VH. The organic carbon ranged from 0.45–2.45% (low to medium) for MP samples, and from 0.34–5.68% (low to high) for VH samples. Overall, the soil samples from MP and VH had < 2% TOC (low to moderately low), except for M11 (2.45%; medium), P3 (3.26%; medium), and P1 (5.68%; high). Although the pecan orchard sites had a slightly higher average %TOC (1.55%) compared to the maize field sites (0.12%), both sampling areas were characterised by a low to moderately low organic carbon content.

Table 7 Total organic carbon content (%) of the maize field and pecan orchard soil samples.

Sample name	TOC (%)	Classification
Mpumalanga province sampling locations		
M1	1.20	Moderately low
M2	0.45	Low
M3	0.47	Low
M4	1.69	Moderately low
M5	2.00	Moderately low
M6	0.52	Low
M7	1.10	Moderately low
M8	1.96	Moderately low
M9	1.95	Moderately low
M10	1.24	Moderately low
M11	2.45	Medium
Vaalharts Valley, Northern Cape province sampling locations		
M12	0.82	Low
M13	0.89	Low
M14	0.49	Low
M15	0.36	Low
P1	5.68	High
P2	0.55	Low
P3	3.26	Medium
P4	0.34	Low

M1–M15: Maize field 1–Maize field 15; P1–P4: Pecan orchard 1–Pecan orchard 4; TOC: total organic carbon content. The %TOC was classified as follows: very low (< 0.05%); low (0.05–1%); moderately low (1–2%); medium (2–4%); and high (> 4%).

4.1.2 Soil particle size distribution

The Endecott dry-sieving method was used to determine the fraction of soil representing clay (< 53 µm); fine sand (53–212 µm); medium sand (212–500 µm); coarse sand (500–2 000 µm); very coarse sand (2 000–4 000 µm); and gravel (> 4 000 µm) (Gerber *et al*., 2015). Sampling locations from the MP were dominated by coarse sand (500–2 000 µm) (**Table 8**). Half of the VH samples contained coarse sand (500–2 000 µm), with the other half classified as fine sand (53–212 µm). Some of the samples were classified as medium sand (212–500 µm; M2 and M7), very coarse sand (2 000–4 000 µm; M8), and gravel (> 4 000 µm; M9 and M10). When comparing soil from the maize fields and pecan orchards with one another, the former mostly contained coarse sand (500–2 000 µm), while the latter predominantly had a smaller grain size (fine sand; 53–212 µm). Overall, the soil from all the sites had a very low (< 2%) clay content (< 53 µm).

Table 8 Soil particle size distribution (% composition) of the maize field and pecan orchard soil samples.

Sample name	% Composition					
	Clay	**Fine sand**	**Medium sand**	**Coarse sand**	**Very coarse sand**	**Gravel**
Mpumalanga province sampling locations						
M1	0.45	10.90	16.88	**35.93**	31.76	4.07
M2	1.04	20.47	**46.13**	29.26	3.11	0.00
M3	0.40	15.31	32.40	**44.59**	2.62	4.69
M4	0.80	14.82	23.39	**36.10**	23.79	1.10
M5	0.45	10.25	22.34	**49.83**	16.49	0.64
M6	1.31	21.39	38.55	**38.70**	0.05	0.00
M7	1.60	24.88	**37.71**	35.43	0.39	0.00
M8	1.73	13.91	14.11	22.15	**26.72**	21.37
M9	0.00	1.77	7.81	29.63	26.51	**34.28**
M10	0.15	5.61	11.92	27.26	26.91	**28.15**
M11	0.30	4.91	11.99	**40.27**	33.60	8.93
Vaalharts Valley, Northern Cape province sampling locations						
M12	0.55	25.62	19.04	**50.98**	3.82	0.00
M13	0.35	27.33	22.55	**45.49**	4.29	0.00
M14	0.54	**46.64**	18.92	27.77	6.13	0.00
M15	0.24	34.79	22.80	**36.83**	53.34	0.00
P1	0.39	**37.05**	36.90	24.84	0.82	0.00
P2	0.75	**45.08**	33.28	18.17	1.66	1.05
P3	0.74	**67.26**	24.81	7.03	0.15	0.00
P4	0.68	36.02	22.60	**36.80**	3.90	0.00

M1–M15: Maize field 1–Maize field 15; P1–P4: Pecan orchard 1–Pecan orchard 4; clay (< 53 µm); fine sand (53–212 µm); medium sand (212–500 µm); coarse sand (500–2 000 µm); very coarse sand (2 000–4 000 µm); and gravel (> 4 000 µm); values in bold indicate the soil fraction with the highest % composition of each sample and which was used to classify the soils.

4.2 *In vitro* bioassays to determine the biological effects associated with agricultural soil

Agrochemicals end up in the environment as complex mixtures. Effects-based methods such as *in vitro* bioassays are a useful tool to assess biological effects such as endocrine disruption, oxidative stress and damage, and acetylcholinesterase inhibition, which occur at the cellular level. In this study, different *in vitro* bioassays were used to assess specific endpoints of the bioavailable fraction of maize and pecan soils: i) reporter gene bioassays were used to investigate xenobiotic metabolism and endocrine disruption; ii) enzymatic and non-enzymatic bioassays were used to determine oxidative stress responses; and iii) an acetylcholinesterase inhibition bioassay was performed to assess damage to non-neuronal cholinergic transmission in detoxification organs.

4.2.1 Reporter gene bioassays

Reporter gene bioassays are useful to investigate nuclear receptor-mediated effects. When a ligand binds to the receptor under investigation it leads to the expression of a reporter gene (*luc*) and the synthesis of a protein (luciferase). When the substrate (luciferin) is added, light is emitted. The amount of luminescence is quantified spectrophotometrically and is directly proportional to the number of ligands present. In this study, three types of reporter gene bioassays assessing different endpoints were used: (i) H4IIE-*luc* bioassay indicative of xenobiotic metabolism through the AhR; (ii) MDA-kb2 bioassay for AR and GR activity; and (iii) T47D-kb*luc* bioassay for ER activity. For each bioassay, the effects concentrations (EC) (EC_{20}, EC_{50}, and EC_{80}; agonism) or inhibitory concentrations (IC) (IC_{20}, IC_{50}, and IC_{80}; antagonism) required to produce 20%, 50% and 80% of the effect of the reference compounds were calculated (Escher *et al*., 2021). In each case, all three values are reported as they are representative of the entire concentration-response curve (Escher *et al*., 2021). Luciferase activity of samples were expressed in terms of each bioassay's positive control (reference compound)—%TCDD Max (AhR activation), %Testosterone Max (AR activation), %Dexamethasone Max (GR activation), %Flutamide Max (AR inhibition), $\%E_2$ Max (ER activation), and %ICI Max (ER inhibition). If a concentration-response curve was obtained for the sample's response, bioanalytical EQs were calculated (TCDD-EQs, testosterone-EQs, dexamethasone-EQs, flutamide-EQs, E_2-EQs, and ICI-EQs). However, if sample responses did not resemble a classical concentration-response curve, fold change was calculated. An $FC > 1$ is considered indicative of activation, while an $FC < 1$ is indicative of inhibition. For all reporter gene bioassays, an MTT cell viability assay was run in parallel to screen the samples for cytotoxicity as quality control (i.e., to ensure that cells were viable throughout the entire bioassay, preventing false-positive results). All cell viability and reporter gene bioassay data are presented as the mean ± standard deviation, with an asterisk (*) indicating statistically significant ($p \leq 0.05$) responses compared to the SC. All *p*-values can be found in **Table S3**.

4.2.1.1 Xenobiotic metabolism

4.2.1.1.1 Aryl hydrocarbon receptor agonism

The H4IIE-*luc* rat hepatoma cell line contains the endogenous AhR and was used to determine whether the samples (1, 3, 9, 28, 83, and 250 mg/mL) contain ligands which activate the AhR. **Figure 19** shows the concentration-response curve obtained for the AhR agonist, TCDD, which was used as the reference compound. The effect concentrations determined for TCDD were as follows: 0.84 pg/mL (EC_{20}), 4.46 pg/mL (EC_{50}), and 23.69 pg/mL (EC_{80}).

The viability of H4IIE-*luc* cells exposed to the samples was compared to the SC (cells dosed with *n*-hexane) which represent 100% cell viability is listed in **Table 9** and interpreted according to the International Organisation for Standardisation (ISO) 10993-5 guideline (ISO, 2009; López-García *et al*., 2014). For the MP samples, most were non-cytotoxic (cell viability ≥ 80%) towards the H4IIE-*luc* cells. However, statistically significant ($p \leq 0.05$) cytotoxicity was observed for the following samples: M2, M3, and M8

(**Table 9**). At the highest concentration of 250 mg/mL soil from M8 was extremely cytotoxic with only 7% cell viability ($p \leq 0.05$). In contrast to this, several of the diluted sample extracts increased cell viability statistically significantly ($p \leq 0.05$): M7 and M10 (**Table 9**). This increase in cell viability was also true for the raw extract of M10. Interestingly, 28 mg/mL of P3 caused statistically significant ($p \leq 0.05$) cytotoxicity (36%) in the H4IIE-*luc* cells, while the two higher concentrations did not (**Table 9**). None of the maize samples from the VH were significantly cytotoxic. Again, there was a sample, P4, of which its raw extract and first dilution increased cell viability statistically significantly (**Table 9**).

Sample responses for AhR activation were expressed in terms of the reference compound as %TCDD Max. The %TCDD Max for MP samples ranged from 1–13%, and from 0–11% for VH samples (**Table 9**). Overall, all the samples had very low %TCDD Max values (all below 20%). Compounds present in these samples did not bind to the AhR. Since none of the samples showed any AhR activation, TCDD-EQs could not be calculated. The SC (*n*-hexane) had a %TCDD Max of 6% which indicates that the solvent did not have any effect on the outcome of the assay.

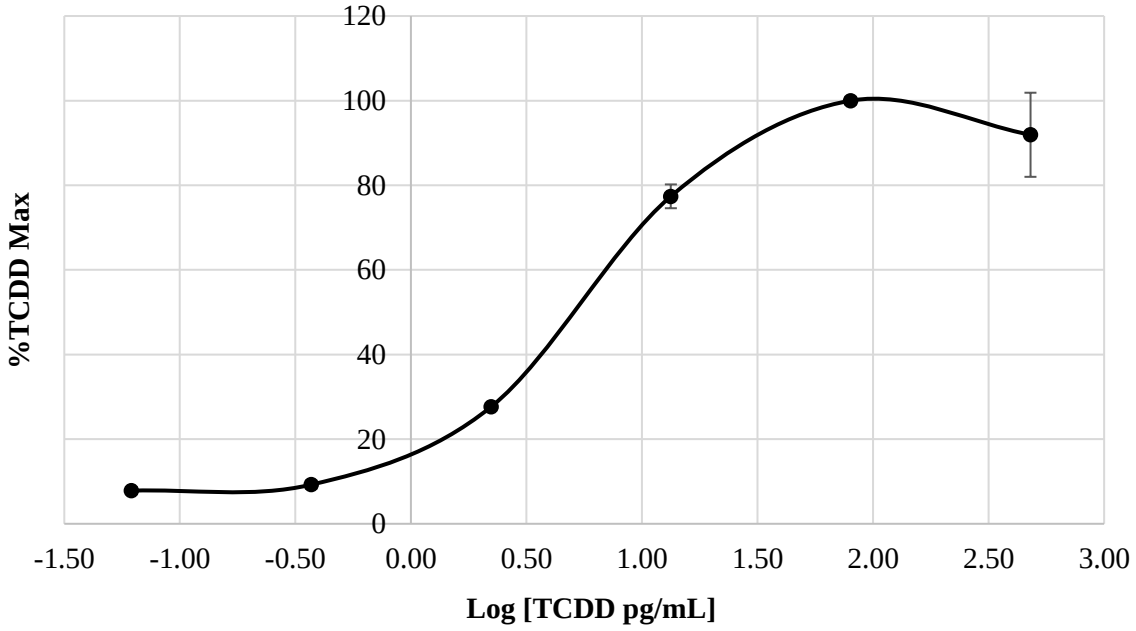

Figure 19 The concentration-response curve obtained for the aryl hydrocarbon receptor activation reference compound, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), during the H4IIE-*luc* bioassay. Luciferase activity is expressed as %TCDD Max against the logarithmically transformed TCDD exposure concentrations (0.06, 0.37, 2.2, 13.3, 80, and 480 pg/mL). Error bars indicate the standard deviation.

Table 9 %TCDD Max and cell viability (%) values of the H4IIE-*luc* cells after exposure to the samples during the assessment of AhR agonism.

Sample name	Exposure concentration (mg/mL)	%TCDD Max	Cell viability (%)
Controls			
BC	N/A	*13 ± 10.03	*73 ± 18.27
NC	N/A	-	*0 ± 6.08
SC	N/A	6 ± 1.79	100 ± 34.80
Mpumalanga province sampling locations			
M1	1	6 ± 1.06	-
	3	8 ± 1.36	-
	9	5 ± 1.19	-
	28	6 ± 1.08	160 ± 63.68
	83	5 ± 1.26	150 ± 56.17
	250	5 ± 1.70	87 ± 43.18
M2	1	6 ± 1.04	-
	3	6 ± 1.87	-
	9	6 ± 1.79	-
	28	5 ± 2.34	158 ± 56.55
	83	5 ± 1.11	83 ± 36.16
	250	*1 ± 0.95	*50 ± 17.64
M3	1	5 ± 0.76	-
	3	6 ± 0.89	-
	9	6 ± 0.67	-
	28	6 ± 0.08	*38 ± 22.24
	83	5 ± 2.20	*35 ± 16.33
	250	6 ± 0.36	98 ± 13.13
M4	1	6 ± 0.29	-
	3	6 ± 0.50	-
	9	7 ± 0.71	-
	28	7 ± 0.22	79 ± 22.00
	83	8 ± 0.33	90 ± 39.14
	250	7 ± 0.63	114 ± 43.46
M5	1	6 ± 0.14	-
	3	6 ± 0.23	-
	9	7 ± 1.09	-
	28	7 ± 0.80	73 ± 6.54
	83	7 ± 0.43	55 ± 17.18
	250	6 ± 0.53	102 ± 44.16
M6	1	7 ± 0.13	-
	3	8 ± 0.50	-
	9	8 ± 0.37	-
	28	*8 ± 0.58	184 ± 24.27
	83	8 ± 1.24	106 ± 21.27

Sample name	Exposure concentration (mg/mL)	%TCDD Max	Cell viability (%)
	250	4 ± 3.21	18 ± 38.36
M7	1	4 ± 0.51	-
	3	5 ± 0.35	-
	9	5 ± 1.28	-
	28	*4 ± 0.56	*214 ± 52.52
	83	*3 ± 0.62	*201 ± 50.25
	250	*1 ± 0.41	138 ± 62.05
M8	1	*3 ± 0.44	-
	3	5 ± 1.07	-
	9	6 ± 2.45	-
	28	*9 ± 1.53	68 ± 25.15
	83	5 ± 2.54	*64 ± 24.31
	250	*2 ± 0.77	*7 ± 10.64
M9	1	*2 ± 0.41	-
	3	*3 ± 0.33	-
	9	5 ± 0.86	-
	28	6 ± 1.07	106 ± 16.31
	83	8 ± 1.73	86 ± 51.32
	250	*10 ± 0.20	75 ± 11.14
M10	1	4 ± 1.59	-
	3	5 ± 1.32	-
	9	5 ± 1.34	-
	28	6 ± 1.65	92 ± 24.91
	83	7 ± 2.50	*116 ± 3.38
	250	6 ± 4.73	*137 ± 19.97
M11	1	5 ± 0.62	-
	3	7 ± 0.51	-
	9	7 ± 0.95	-
	28	*8 ± 0.71	84 ± 18.72
	83	*9 ± 1.53	90 ± 16.90
	250	6 ± 1.17	99 ± 19.95
Vaalharts Valley, Northern Cape province sampling locations			
M12	1	6 ± 1.37	-
	3	6 ± 0.89	-
	9	7 ± 1.99	-
	28	*8 ± 0.91	120 ± 10.03
	83	8 ± 0.92	98 ± 18.75
	250	*4 ± 1.13	74 ± 35.14
M13	1	7 ± 0.67	-
	3	7 ± 0.29	-
	9	8 ± 0.07	-
	28	7 ± 1.43	106 ± 32.84
	83	*2 ± 3.05	96 ± 44.32

Sample name	Exposure concentration (mg/mL)	%TCDD Max	Cell viability (%)
	250	3 ± 2.88	63 ± 16.94
M14	1	*3 ± 0.29	-
	3	5 ± 0.31	-
	9	4 ± 0.99	-
	28	*2 ± 0.99	128 ± 14.76
	83	*3 ± 0.44	111 ± 23.25
	250	*0 ± 0.03	70 ± 12.41
M15	1	7 ± 1.18	-
	3	*8 ± 0.65	-
	9	*8 ± 0.43	-
	28	*11 ± 0.65	105 ± 3.01
	83	*9 ± 0.55	87 ± 9.73
	250	6 ± 0.47	99 ± 10.44
P1	1	5 ± 0.03	-
	3	7 ± 0.48	-
	9	*8 ± 0.90	-
	28	*9 ± 0.41	131 ± 55.20
	83	*9 ± 0.56	98 ± 28.39
	250	*8 ± 0.70	*68 ± 15.18
P2	1	6 ± 0.98	-
	3	7 ± 0.33	-
	9	8 ± 1.95	-
	28	*11 ± 0.55	147 ± 35.84
	83	7 ± 0.12	122 ± 28.77
	250	6 ± 0.42	*77 ± 4.93
P3	1	8 ± 0.70	-
	3	*8 ± 0.92	-
	9	*8 ± 0.24	-
	28	*9 ± 0.64	*36 ± 28.79
	83	8 ± 0.33	100 ± 46.67
	250	5 ± 0.98	77 ± 35.53
P4	1	*8 ± 0.32	-
	3	*8 ± 0.35	-
	9	*8 ± 0.36	-
	28	*9 ± 0.28	78 ± 7.53
	83	*9 ± 0.56	*138 ± 7.15
	250	6 ± 0.10	*171 ± 19.91
Non-cytotoxic (≥ 80%)	Weakly cytotoxic (60–79%)	Moderately cytotoxic (41–59%)	Strongly cytotoxic (≤ 40%)

The cytotoxic effect on viability was interpreted according to ISO (2009). All data is presented as mean ± standard deviation. *Statistically significant compared to the SC ($p \leq 0.05$); -cytotoxicity of concentration was not investigated; BC: blank control (untreated cells); M1–M15: Maize field 1–Maize field 15; N/A: not applicable; NC: negative control (cells treated with methanol, i.e., non-viable cells); P1–P4: Pecan orchard 1–Pecan orchard 4; SC: solvent control (cells dosed with *n*-hexane, i.e., viable cells).

4.2.1.2 Endocrine disruption

4.2.1.2.1 Androgen and glucocorticoid receptor agonism

The MDA-kb2 breast cancer cell line expresses both the AR and GR. Therefore, a second bioassay is performed in the event of receptor agonism. The first bioassay only assesses activation and if activation is observed a second bioassay is performed to distinguish whether the activation occurred due to AR- or GR-mediated ligands. During the second bioassay, a known AR agonist, such as flutamide, which blocks the AR but not the GR, is co-administered. If any receptor binding of samples occurs during this second bioassay, it is attributed to the presence of GR agonists (GR activation). MDA-kb2 cells were exposed to samples (1, 3, 9, 28, 83, and 250 mg/mL) and testosterone was used as the reference compound for AR agonism. Based on the concentration-response curve obtained for testosterone (**Figure 20**) the following effects concentrations were calculated: 40.05 pg/mL (EC_{20}), 113.10 pg/mL (EC_{50}), and 319.42 pg/mL (EC_{80}).

Table 10 lists the viability of MDA-kb2 cells exposed to the sample compared to the SC (cells dosed with methanol) which represent 100% cell viability. Strong cytotoxicity (≤ 40% cell viability) ($p \leq 0.05$) was observed for several of the MP samples: M1 (250 mg/mL; strongly cytotoxic), M3 (28 mg/mL; strongly cytotoxic), M4 (250 mg/mL; strongly cytotoxic), and M10 (28 mg/mL; strongly cytotoxic). Interestingly, all M5 exposure concentrations were strongly cytotoxic ($p \leq 0.05$) with 0% cell viability reported for 28, 83, and 250 mg/mL. For M11, 83 mg/mL significantly enhanced ($p \leq 0.05$) cell viability (287%). The majority of the VH samples were weakly to non-cytotoxic, except for M14 (250 mg/mL; strongly cytotoxic) and P4 (83 and 250 mg/mL; moderately cytotoxic). Although some VH samples (M12, M13, and P1) increased the cell viability of the MDA-kb2 cell line above 100%, these responses were not statistically significant ($p \leq 0.05$) compared to the SC.

Sample responses for AR activation were expressed in terms of the reference compound as %Testosterone Max. The %Testosterone Max for MP samples ranged from 3–18%, and from 3–19% for VH samples (**Table 10**). Generally, all the samples had %Testosterone Max values below 20%, indicating no AR agonistic effects. Since no AR activation was observed for any of the samples, testosterone-EQs could not be calculated. The SC (methanol) had a %Testosterone Max of 11% which indicates that the solvent did not have any effect on the outcome of the assay. Seeing as no activation was observed for the samples, a second bioassay was not performed, and GR activation was not further investigated.

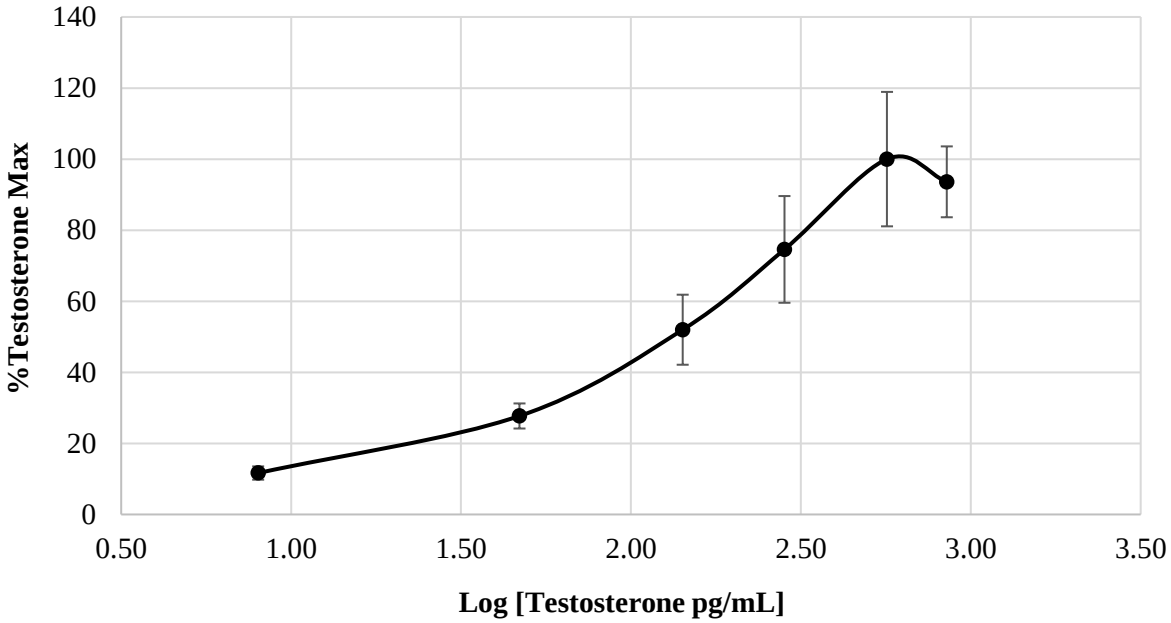

Figure 20 The concentration-response curve obtained for the androgen receptor activation reference compound, testosterone, during the MDA-kb2 bioassay. Luciferase activity is expressed as %Testosterone Max against the logarithmically transformed testosterone exposure concentrations (8, 47, 142, 283, 567, and 850 pg/mL). Error bars indicate the standard deviation**.**

Table 10 %Testosterone Max and cell viability (%) values of the MDA-kb2 cells after exposure to the samples during the assessment of AR agonism.

Sample name	Exposure concentration (mg/mL)	%Testosterone Max	Cell viability (%)
Controls			
BC	N/A	*12 ± 2.42	100 ± 15.87
NC	N/A	-	*0 ± 14.75
SC	N/A	11 ± 2.49	100 ± 7.54
Mpumalanga province sampling locations			
M1	1	*18 ± 1.79	-
	3	*13 ± 0.42	-
	9	*16 ± 1.53	-
	28	*16 ± 5.28	115 ± 87.57
	83	14 ± 8.53	249 ± 26.52
	250	*3 ± 0.15	*18 ± 35.44
M2	1	*17 ± 1.84	-
	3	*14 ± 2.12	-
	9	*14 ± 0.25	-
	28	*14 ± 0.72	130 ± 82.95
	83	11 ± 1.28	123 ± 60.16
	250	10 ± 0.17	133 ± 10.61
M3	1	11 ± 1.15	-

Sample name	Exposure concentration (mg/mL)	%Testosterone Max	Cell viability (%)
	3	11 ± 1.17	-
	9	11 ± 0.48	-
	28	12 ± 1.82	*38 ± 11.46
	83	12 ± 2.08	70 ± 45.83
	250	11 ± 1.09	70 ± 37.75
M4	1	12 ± 1.54	-
	3	12 ± 0.81	-
	9	*13 ± 0.28	-
	28	12 ± 1.78	80 ± 15.00
	83	12 ± 1.73	*43 ± 7.50
	250	12 ± 0.75	*40 ± 15.61
M5	1	12 ± 1.59	-
	3	12 ± 0.34	-
	9	11 ± 2.01	-
	28	11 ± 0.99	*0.00 ± 33.82
	83	10 ± 1.99	*0.00 ± 42.65
	250	*8 ± 0.38	*0.00 ± 31.22
M6	1	10 ± 1.09	-
	3	11 ± 0.67	-
	9	10 ± 1.22	-
	28	11 ± 2.79	150 ± 58.67
	83	*7 ± 2.09	157 ± 64.57
	250	*3 ± 0.51	35 ± 40.62
M7	1	*14 ± 0.65	-
	3	13 ± 0.89	-
	9	13 ± 3.69	-
	28	*17 ± 3.27	105 ± 49.65
	83	*14 ± 0.39	72 ± 55.77
	250	12 ± 3.41	77 ± 33.78
M8	1	13 ± 2.66	-
	3	13 ± 2.97	-
	9	12 ± 1.59	-
	28	*14 ± 2.13	119 ± 57.92
	83	*15 ± 0.83	83 ± 19.15
	250	*15 ± 1.66	83 ± 9.03
M9	1	11 ± 1.15	-
	3	13 ± 1.35	-
	9	12 ± 4.24	-
	28	9 ± 4.46	57 ± 11.06
	83	13 ± 2.85	38 ± 25.53
	250	10 ± 3.27	68 ± 19.50
M10	1	12 ± 0.94	-
	3	12 ± 1.00	-

Sample name	Exposure concentration (mg/mL)	%Testosterone Max	Cell viability (%)
	9	12 ± 2.24	-
	28	*16 ± 0.96	*17 ± 9.75
	83	10 ± 1.01	100 ± 59.31
	250	*14 ± 1.90	109 ± 23.01
M11	1	12 ± 0.57	-
	3	11 ± 0.48	-
	9	13 ± 0.54	-
	28	*13 ± 0.49	183 ± 45.13
	83	8 ± 5.89	*287 ± 51.59
	250	*5 ± 1.02	540 ± 81.24
Vaalharts Valley, Northern Cape province sampling locations			
M12	1	10 ± 0.84	-
	3	10 ± 0.58	-
	9	10 ± 0.57	-
	28	10 ± 0.72	343 ± 63.19
	83	9 ± 1.96	209 ± 45.13
	250	9 ± 2.07	145 ± 63.18
M13	1	16 ± 5.39	-
	3	*16 ± 1.50	-
	9	*17 ± 1.82	-
	28	*14 ± 2.51	96 ± 19.50
	83	13 ± 0.36	106 ± 27.08
	250	8 ± 3.51	151 ± 25.53
M14	1	*19 ± 3.04	-
	3	*17 ± 2.02	-
	9	*18 ± 1.14	-
	28	*17 ± 1.69	36 ± 25.53
	83	12 ± 2.85	15 ± 44.83
	250	9 ± 2.87	*19 ± 7.37
M15	1	11 ± 0.90	-
	3	12 ± 0.80	-
	9	*14 ± 0.94	-
	28	13 ± 2.18	94 ± 45.13
	83	*17 ± 0.59	74 ± 71.08
	250	9 ± 3.70	194 ± 72.59
P1	1	11 ± 0.83	-
	3	13 ± 0.83	-
	9	11 ± 0.92	-
	28	11 ± 0.84	112 ± 27.16
	83	11 ± 1.71	137 ± 57.24
	250	*8 ± 0.93	129 ± 28.48
P2	1	11 ± 2.64	-
	3	13 ± 2.01	-

Sample name	Exposure concentration (mg/mL)	%Testosterone Max	Cell viability (%)
	9	10 ± 1.28	-
	28	13 ± 1.11	142 ± 50.86
	83	12 ± 1.36	*72 ± 10.48
	250	*3 ± 4.43	64 ± 51.40
P3	1	10 ± 1.36	-
	3	13 ± 1.34	-
	9	12 ± 0.91	-
	28	*16 ± 4.03	142 ± 50.86
	83	9 ± 3.29	*72 ± 10.48
	250	*4 ± 0.58	64 ± 51.40
P4	1	*8 ± 0.74	-
	3	*8 ± 1.03	-
	9	*8 ± 0.92	-
	28	*16 ± 2.18	104 ± 30.34
	83	*16 ± 1.02	*59 ± 10.28
	250	14 ± 3.17	*49 ± 8.67
Non-cytotoxic (≥ 80%)	Weakly cytotoxic (60–79%)	Moderately cytotoxic (41–59%)	Strongly cytotoxic (≤ 40%)

The cytotoxic effect on viability was interpreted according to ISO (2009). All data is presented as mean ± standard deviation. *Statistically significant compared to the SC ($p \leq 0.05$); -Cytotoxicity of concentration was not investigated; BC: blank control (untreated cells); M1–M15: Maize field 1–Maize field 15; N/A: not applicable; NC: negative control (cells treated with methanol, i.e., non-viable cells); P1–P4: Pecan orchard 1–Pecan orchard 4; SC: solvent control (cells dosed with methanol, i.e., viable cells).

4.2.1.2.2 Androgen receptor antagonism

To determine whether the samples exhibit any anti-androgenic effects, the MDA-kb2 cells were exposed to the samples containing a background concentration of testosterone (0.283 ng/mL). This was done to ensure 80% activation of the AR. A concentration-response curve was obtained for the AR antagonist, flutamide, which served as the reference compound for anti-androgenic effects (**Figure 21**). The following inhibitory concentrations of flutamide were determined: 0.14 µg/mL (IC_{20}), 0.66 µg/mL (IC_{50}), and 3.08 µg/mL (IC_{80}).

Since cytotoxicity can mask receptor-mediated effects, the inclusion of an MTT cell viability assay for quality control is especially important for bioassay in which antagonism is assessed. Viable cells validate that low %Flutamide Max values are indicative of receptor inhibition and not due to dead cells. There were some samples where cytotoxicity ($p \leq 0.05$) was observed for the raw extracts: M3 and P3. An interesting observation was that in the case of M2 and P2 the dilutions were cytotoxic but not the raw extracts. Cell viability and %Flutamide Max values could not be determined for the highest exposure concentration of M2 and P2 due to the presence of bacteria in the wells. Since both the MTT and MDA-kb2 bioassays had bacteria, the raw extract of M2 and P2 were likely cytotoxic, resulting in cell death and subsequent bacterial contamination. Although the highest concentration (250 mg/mL) of M3 was strongly cytotoxic ($p \leq 0.05$),

it did not seem to have affected the %Flutamide Max value obtained. When comparing the 87 %Flutamide Max of M3 to the graph of the flutamide concentration-response curve (**Figure 21**), there is confirmation that still much light was emitted by the cells. Exposure to the raw extract and dilutions of several samples caused an increase in cell viability ($p \leq 0.05$) to above 100% in the MDA-kb2 cell line, including M9–M15 (**Table 11**). Since the BC had a cell viability of 83% the increase in cell viability above 100% of the aforementioned samples was not attributed to the background of testosterone that the cells received. In other words, the additional testosterone did not cause enhanced cell growth of the MDA-kb2 cells. Rather, the effects are due to other compounds present in these samples.

Sample responses for AR antagonism were expressed in terms of the reference compound as %Flutamide Max. The %Flutamide Max values for MP samples ranged from 123–27% and for VH from 153–3% (**Table 11**). %Flutamide Max values were low for M5, P2, and P3 (**Table 11**) which can be attributed to the cytotoxicity observed for the corresponding concentrations and not due AR inhibition. There was evidence of potential anti-androgenic effects for some of the MP (M1, M3, M4, M6, M7, M8, M9, and M10) and VH (M13, M15, and P4) samples. However, since these samples did not exhibit a classical concentration-response curve (as shown in **Figure 21**), only FC values could be calculated. All the previously mentioned samples had an FC < 1, supporting the observation of AR inhibition (**Figure 22**). Overall, eight of the ten samples showed statistically significant ($p \leq 0.05$) responses: M1, M4, M7, M8, M9, M10, M13, and P4. Maize 8 had the lowest FC (0.31). However, it is important to note that evidence of antagonism should be interpreted with caution since cytotoxicity can mask antagonistic effects. Any evidence of cytotoxicity (even weak cytotoxicity) can affect the results obtained for antagonism. The SC (methanol) had a %Flutamide Max of 103% which indicates that the solvent did not have any effect on the outcome of the assay.

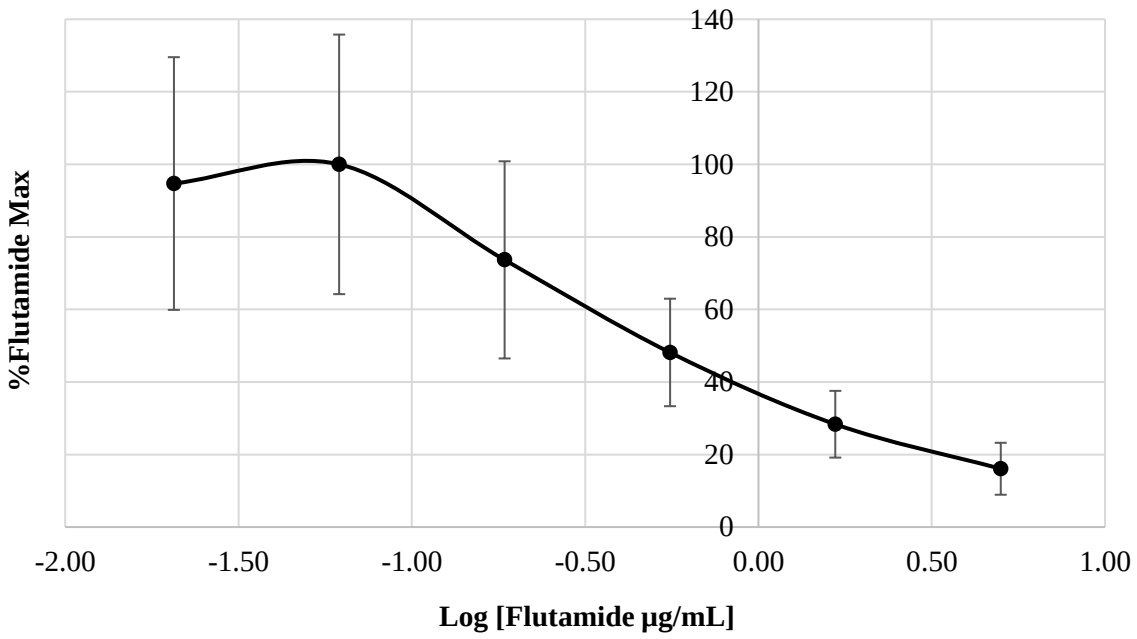

Figure 21 The concentration-response curve obtained for the androgen receptor (AR) inhibition reference compound, flutamide, during the MDA-kb2 bioassay. These cells received a background of the AR agonist, testosterone (0.283 ng/mL), to ensure 80% activation of the AR. Luciferase activity is expressed as %Flutamide Max against the logarithmically transformed flutamide exposure concentrations (0.02, 0.06, 0.19, 0.56, 1.67, and 5.0 µg/mL). Error bars indicate the standard deviation.

Table 11 %Flutamide Max and cell viability (%) values of the MDA kb2 cells after exposure to the samples during the assessment of AR antagonism

Sample name	Exposure concentration (mg/mL)	%Flutamide Max	Cell viability (%)
Controls			
BC	N/A	91 ± 35.91	83 ± 16.07
NC	N/A	-	*0 ± 4.76
SC	N/A	103 ± 38.67	100 ± 7.65
Mpumalanga province sampling locations			
M1	1	*53 ± 2.11	-
	3	*54 ± 23.28	-
	9	*55 ± 16.10	-
	28	*37 ± 2.67	76 ± 11.36
	83	*27 ± 5.94	76 ± 23.27
	250	*44 ± 18.85	84 ± 10.34
M2	1	*66 ± 10.74	-
	3	77 ± 8.59	-
	9	76 ± 12.85	-
	28	*64 ± 2.56	*35 ± 13.47
	83	*59 ± 7.25	93 ± 28.14

Sample name	Exposure concentration (mg/mL)	%Flutamide Max	Cell viability (%)
	250	87 ± 11.78	†
M3	1	*66 ± 10.74	-
	3	77 ± 8.59	-
	9	76 ± 12.85	-
	28	*64 ± 2.56	75 ± 56.91
	83	*59 ± 7.25	80 ± 21.19
	250	87 ± 11.78	*49 ± 9.64
M4	1	97 ± 5.62	-
	3	109 ± 19.56	-
	9	104 ± 25.33	-
	28	91 ± 14.44	88 ± 19.66
	83	81 ± 6.12	89 ± 23.16
	250	*65 ± 9.09	105 ± 27.90
M5	1	*54 ± 4.72	-
	3	*61 ± 19.37	-
	9	*57 ± 8.64	-
	28	*61 ± 5.46	81 ± 14.87
	83	*34 ± 0.51	57 ± 18.15
	250	*26 ± 3.16	54 ± 11.19
M6	1	*57 ± 16.27	-
	3	*60 ± 2.15	-
	9	70 ± 9.92	-
	28	*53 ± 14.19	89 ± 23.50
	83	*29 ± 2.92	79 ± 17.94
	250	*50 ± 3.35	82 ± 31.50
M7	1	*52 ± 15.11	-
	3	81 ± 19.81	-
	9	*67 ± 13.20	-
	28	*61 ± 7.98	*74 ± 0.45
	83	*48 ± 13.07	*72 ± 1.34
	250	*49 ± 7.59	80 ± 14.32
M8	1	*46 ± 7.11	-
	3	78 ± 25.80	-
	9	79 ± 27.73	-
	28	57 ± 27.05	*75 ± 8.51
	83	*46 ± 4.24	87 ± 19.82
	250	*24 ± 5.80	79 ± 19.52
M9	1	105 ± 12.45	-
	3	109 ± 35.99	-
	9	97 ± 13.55	-
	28	105 ± 14.95	*118 ± 2.73
	83	85 ± 9.07	118 ± 20.85
	250	*61 ± 8.65	79 ± 17.12

Sample name	Exposure concentration (mg/mL)	%Flutamide Max	Cell viability (%)
M10	1	123 ± 33.477	-
	3	115 ± 16.87	-
	9	111 ± 16.46	-
	28	97 ± 15.41	103 ± 4.32
	83	119 ± 17.37	*119 ± 10.27
	250	*69 ± 3.58	92 ± 2.73
M11	1	83 ± 4.49	-
	3	77 ± 5.20	-
	9	80 ± 7.39	-
	28	87 ± 15.21	*149 ± 23.98
	83	72 ± 11.41	*153 ± 35.44
	250	*71 ± 5.76	122 ± 16.25
Vaalharts Valley, Northern Cape province sampling locations			
M12	1	73 ± 18.34	-
	3	76 ± 10.79	-
	9	69 ± 18.18	-
	28	70 ± 5.81	124 ± 28.64
	83	75 ± 11.75	*127 ± 12.41
	250	73 ± 8.14	*202 ± 18.49
M13	1	91 ± 16.76	-
	3	134 ± 25.04	-
	9	93 ± 12.47	-
	28	89 ± 9.97	126 ± 15.61
	83	95 ± 24.77	112 ± 14.94
	250	76 ± 13.76	93 ± 18.73
M14	1	138 ± 20.88	-
	3	*138 ± 10.65	-
	9	129 ± 8.36	-
	28	115 ± 4.05	83 ± 26.56
	83	136 ± 27.59	104 ± 12.65
	250	*147 ± 12.41	*183 ± 12.83
M15	1	103 ± 12.52	-
	3	93 ± 17.94	-
	9	72 ± 17.47	-
	28	87 ± 12.36	122 ± 60.85
	83	92 ± 3.13	97 ± 6.33
	250	*66 ± 8.94	93 ± 36.68
P1	1	114 ± 25.11	-
	3	124 ± 14.36	-
	9	93 ± 25.60	-
	28	105 ± 33.72	69 ± 18.83
	83	117 ± 5.16	66 ± 20.24
	250	69 ± 12.55	61 ± 16.67

Sample name	Exposure concentration (mg/mL)	%Flutamide Max	Cell viability (%)
P2	1	*52 ± 9.93	-
	3	*58 ± 19.40	-
	9	*50 ± 13.21	-
	28	*45 ± 8.56	*28 ± 8.93
	83	*32 ± 12.06	*44 ± 13.37
	250	†	†
P3	1	*63 ± 6.70	-
	3	70 ± 14.08	-
	9	*54 ± 4.07	-
	28	*60 ± 8.47	*61 ± 4.07
	83	*44 ± 14.94	72 ± 21.31
	250	*32 ± 2.35	*52 ± 11.25
P4	1	125 ± 17.68	-
	3	114 ± 11.80	-
	9	*153 ± 24.58	-
	28	115 ± 28.60	65 ± 40.48
	83	86 ± 18.43	93 ± 32.35
	250	73 ± 5.23	76 ± 26.55
Non-cytotoxic (≥ 80%)	Weakly cytotoxic (60–79%)	Moderately cytotoxic (41–59%)	Strongly cytotoxic (≤ 40%)

The cytotoxic effect on viability was interpreted according to ISO (2009). All data is presented as mean ± standard deviation. *Statistically significant compared to the SC ($p \leq 0.05$); -cytotoxicity of concentration was not investigated; †cell viability (%) could not be determined due to the presence of bacteria in the wells of the 96-well cell culture test plate; BC: blank control (untreated cells); M1–M15: Maize field 1–Maize field 15; N/A: not applicable; NC: negative control (cells treated with methanol, i.e., non-viable cells); P1–P4: Pecan orchard 1–Pecan orchard 4; SC: solvent control (cells dosed with methanol, i.e., viable cells).

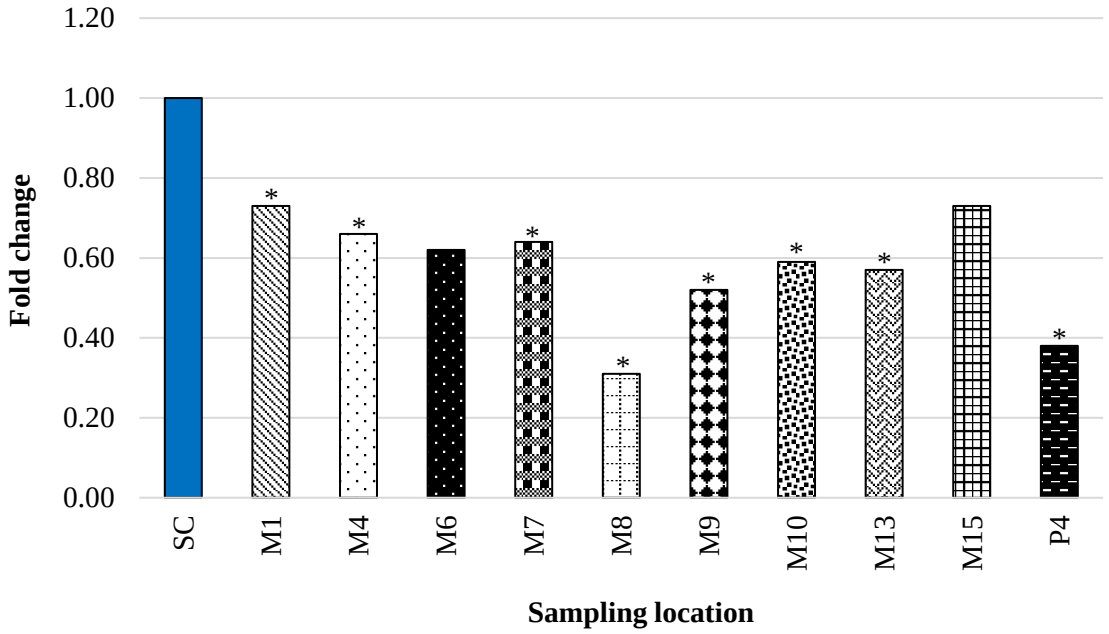

Figure 22 Fold change values calculated for anti-androgenic activity of samples at 250 mg/mL. Sample responses were compared to the solvent control (SC) (cells dosed with methanol, i.e., viable cells). *Statistically significantly ($p \leq 0.05$) lower compared to the SC; M1, M4, M6, M7, M8, M10, M13, and M15: Maize fields 1, 4, 6, 7, 8, 10, 13, and 15; P4: Pecan orchard 4.

4.2.1.2.3 Oestrogen receptor agonism

The T47D-kb*luc* breast cancer cell line endogenously expresses the ER and was used to determine the oestrogenic activity of the samples (1, 3, 9, 28, 83, and 250 mg/mL). 17β-Oestradiol was used as the reference compound for ER agonism, and the resulting concentration-curve obtained is shown in **Figure 23**. For the E_2 concentration-response curve the following effect concentrations were determined: 0.27 pg/mL (EC_{20}), 0.63 pg/mL (EC_{50}), and 1.43 pg/mL (EC_{80}).

Compared to the SC (cells dosed with ethanol) which represent 100% viable cells, the T47D-KB*luc* cell line's viability ranged from 60–187% and 84–132% after exposure to the MP and VH samples, respectively (**Table 12**). The highest concentration (250 mg/mL) of M2 was only weakly cytotoxic (60%, $p \leq 0.05$), with most of the samples not causing cytotoxicity in the T47D-KB*luc* cell line, with high cell viability (> 80%) reported overall. Three samples from MP significantly ($p \leq 0.05$) increased cell viability above 100%: M2, M3, and M4 (**Table 12**).

Responses for ER agonism were expressed in terms of the reference compound as $\%E_2$ Max (**Table 12**). As mentioned in Chapter 2, we experienced a background of E_2 in our tissue culture laboratory during the ER activation bioassay. T47D-KB*luc* bioassay for ER agonism specifically was repeated several times with the reference compound (E_2) to obtain a concentration-response curve that covers the entire range of responses

(i.e., > 20% to 100% of E_2 Max) (results not shown). However, this could not be accomplished and in an attempt to minimise the effect of oestrogenic contamination on the outcome of the ER activation assay, the E_2 concentration range was prepared in nutrient medium which contained a background concentration of the ER antagonist, ICI (0.04 ng/mL). However, $\%E_2$ Max values below 20% could still not be obtained. It is evident from the E_2 concentration-response curve (**Figure 23**) that although the E_2 concentration decreases, the amount of light emitted (luciferase activity) for the lowest three concentrations (0.02, 0.11, and 0.39 pg/mL) remains almost constant, resulting in $\%E_2$ Max values of 29% (0.02 pg/mL), 30% (0.11 pg/mL) and 31% (0.39 pg/mL). Moreover, the $\%E_2$ Max for the BC (untreated cells) and SC (cells dosed with ethanol) were 28% and 27%, respectively (**Table 12**). This indicates that the T47D-KB*luc* cells are naturally excited due to an unknown source of oestrogen in our tissue culture laboratory.

The $\%E_2$ Max values for the samples ranged from 3–26% (MP) and 8–48% (VH). Although the T47D-KB*luc* bioassay experienced a background of E_2 (~30%), the majority of the samples did not show any oestrogenic effects with an overall $\%E_2$ Max of < 20%. A few VH samples (M12, M13, P2, and P3) showed evidence of potential ER activation. However, these responses did not resemble a classical concentration-response curve (as shown in **Figure 23**) and as a result E_2-EQs could not be calculated. Rather, FC was calculated for the sample concentrations with the highest $\%E_2$ Max value (**Figure 24**). All these samples (M12, M13, P2, and P3) had an FC > 1, supporting potential oestrogenic effects. Responses were statistically significant ($p \leq 0.05$) for M13, P2, and P3. Maize 13 had the highest FC (1.77).

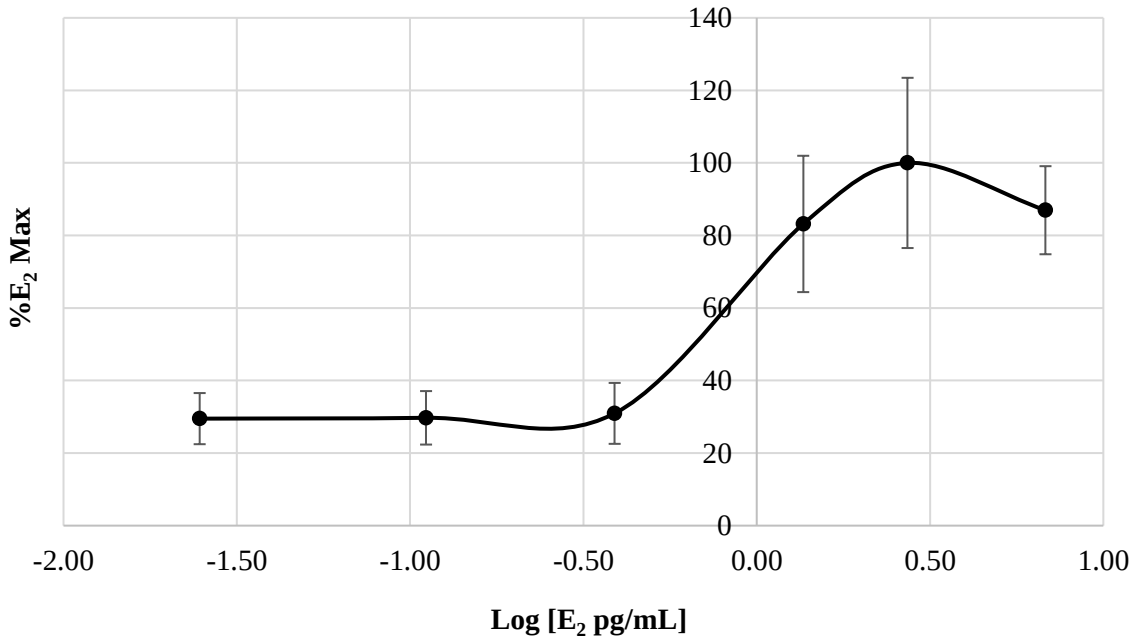

Figure 23 The concentration-response curve obtained for the oestrogen receptor (ER) activation reference compound, 17β-oestradiol (E_2), during the T47D-KB*luc* bioassay. These cells received a background of the ER antagonist, ICI (0.04 ng/mL), in an attempt to minimise the effect of the oestrogen background experienced in our laboratory. Luciferase activity is expressed as $\%E_2$ Max against the logarithmically transformed E_2 exposure concentrations (0.02, 0.11, 0.39, 1.36, 2.72, and 6.81 pg/mL). Error bars indicate the standard deviation.

Table 12 %E_2 Max values and cell viability (%) values of the T47D-KB*luc* cells after exposure to the samples during the assessment of ER agonism.

Sample name	Exposure concentration (mg/mL)	%E_2 Max	Cell viability (%)
Controls			
BC	N/A	28 ± 8.71	84 ± 27.47
NC	N/A	-	*0 ± 0.76
SC	N/A	27 ± 9.76	100 ± 23.11
Mpumalanga province sampling locations			
M1	1	25 ± 3.35	-
	3	21 ± 2.62	-
	9	32 ± 13.51	-
	28	22 ± 5.24	124 ± 36.18
	83	*16 ± 1.52	108 ± 23.00
	250	*3 ± 0.28	79 ± 24.02
M2	1	20 ± 2.89	-
	3	20 ± 1.39	-
	9	20 ± 3.05	-
	28	26 ± 4.00	*158 ± 20.81
	83	*18 ± 0.79	129 ± 30.46
	250	*6 ± 0.72	*60 ± 6.45
M3	1	21 ± 1.68	-
	3	*18 ± 1.25	-
	9	*16 ± 1.63	-
	28	*16 ± 0.85	81 ± 12.76
	83	*12 ± 1.26	75 ± 13.87
	250	*7 ± 1.01	*187 ± 26.66
M4	1	*15 ± 1.56	-
	3	*16 ± 1.97	-
	9	*15 ± 1.58	-
	28	*13 ± 1.32	101 ± 22.18
	83	*9 ± 0.85	127 ± 42.68
	250	*5 ± 0.87	*174 ± 7.81
M5	1	*14 ± 1.47	-
	3	*13 ± 1.00	-
	9	*13 ± 0.07	-
	28	*12 ± 0.72	98 ± 51.14
	83	*10 ± 1.03	130 ± 33.58
	250	*7 ± 0.43	121 ± 9.47
M6	1	*15 ± 0.64	-
	3	*18 ± 0.98	-
	9	*15 ± 0.76	-
	28	*19 ± 1.34	*78 ± 7.99
	83	*13 ± 0.41	88 ± 19.65

Sample name	Exposure concentration (mg/mL)	%E_2 Max	Cell viability (%)
	250	*8 ± 0.25	*81 ± 13.60
M7	1	*19 ± 0.64	-
	3	*17 ± 0.22	-
	9	*17 ± 0.85	-
	28	*16 ± 0.56	92 ± 37.89
	83	*16 ± 1.61	89 ± 15.89
	250	*7 ± 1.32	103 ± 2.36
M8	1	*17 ± 1.04	-
	3	*17 ± 1.18	-
	9	*18 ± 1.58	-
	28	*18 ± 2.14	93 ± 23.67
	83	*13 ± 1.02	78 ± 14.46
	250	*8 ± 1.30	94 ± 5.36
M9	1	22 ± 1.48	-
	3	24 ± 2.39	-
	9	22 ± 1.17	-
	28	*17 ± 0.30	100 ± 6.38
	83	*13 ± 0.31	105 ± 14.98
	250	*10 ± 0.65	92 ± 4.61
M10	1	22 ± 1.48	-
	3	24 ± 2.39	-
	9	22 ± 1.17	-
	28	*17 ± 0.30	*82 ± 4.02
	83	*13 ± 0.31	91 ± 18.09
	250	*10 ± 0.65	113 ± 9.62
M11	1	22 ± 0.72	-
	3	22 ± 0.83	-
	9	22 ± 2.53	-
	28	22 ± 2.60	113 ± 3.17
	83	23 ± 0.78	115 ± 0.91
	250	*18 ± 0.68	100 ± 22.01
Vaalharts Valley, Northern Cape province sampling locations			
M12	1	*18 ± 3.28	-
	3	*19 ± 2.13	-
	9	*19 ± 1.08	-
	28	27 ± 1.22	116 ± 35.85
	83	34 ± 1.82	84 ± 3.47
	250	*8 ± 0.52	119 ± 21.42
M13	1	*16 ± 1.08	-
	3	*15 ± 1.19	-
	9	*14 ± 1.31	-
	28	*48 ± 3.25	132 ± 19.23
	83	*15 ± 3.75	94 ± 11.33

Sample name	Exposure concentration (mg/mL)	%E_2 Max	Cell viability (%)
	250	*8 ± 1.58	113 ± 34.87
M14	1	*16 ± 1.46	-
	3	*17 ± 0.97	-
	9	*15 ± 3.10	-
	28	19 ± 3.60	101 ± 15.65
	83	*18 ± 1.94	111 ± 34.85
	250	*17 ± 2.51	112 ± 21.83
M15	1	29 ± 3.91	-
	3	30 ± 0.78	-
	9	30 ± 1.30	-
	28	29 ± 1.70	122 ± 7.90
	83	*20 ± 1.48	121 ± 41.23
	250	*8 ± 0.42	102 ± 4.36
P1	1	24 ± 4.45	-
	3	29 ± 2.73	-
	9	31 ± 3.64	-
	28	28 ± 3.64	87 ± 18.13
	83	24 ± 2.76	86 ± 25.23
	250	*17 ± 3.38	99 ± 9.24
P2	1	26 ± 2.88	-
	3	*41 ± 2.71	-
	9	34 ± 7.35	-
	28	29 ± 3.59	98 ± 16.81
	83	25 ± 3.58	117 ± 16.81
	250	*14 ± 1.41	105 ± 13.44
P3	1	24 ± 2.26	-
	3	26 ± 2.47	-
	9	31 ± 1.95	-
	28	34 ± 2.89	87 ± 15.20
	83	*38 ± 2.94	107 ± 34.13
	250	*38 ± 0.49	92 ± 10.71
P4	1	*11 ± 1.04	-
	3	*13 ± 2.78	-
	9	*19 ± 0.57	-
	28	*17 ± 1.71	105 ± 17.00
	83	*16 ± 1.14	96 ± 28.49
	250	*10 ± 1.16	97 ± 11.79
Non-cytotoxic (≥ 80%)	Weakly cytotoxic (60–79%)	Moderately cytotoxic (41–59%)	Strongly cytotoxic (≤ 40%)

The cytotoxic effect on viability was interpreted according to ISO (2009). All data is presented as mean ± standard deviation. *Statistically significant compared to the SC ($p \leq 0.05$); -cytotoxicity of concentration was not investigated; †cell viability (%) could not be determined due to the presence of bacteria in the wells of the 96-well cell culture test plate; BC: blank control (untreated cells); M1–M15: Maize field 1–Maize field 15; N/A: not applicable; NC: negative control (cells treated with methanol, i.e., non-viable cells); P1–P4: Pecan orchard 1–Pecan orchard 4; SC: solvent control (cells dosed with ethanol, i.e., viable cells).

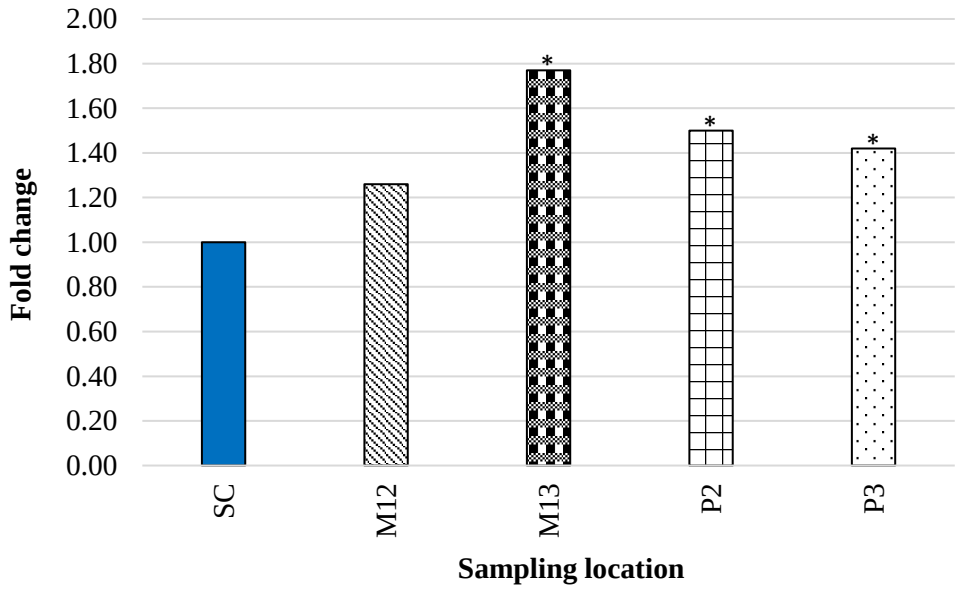

Figure 24 Fold change values calculated for agonistic oestrogen activity of samples. Sample responses were compared to the solvent control (SC) (cells dosed with ethanol, i.e., viable cells). *Statistically significantly ($p \leq 0.05$) higher compared to the SC; M12–M13: Maize fields 12–13; P2–P3: Pecan orchards 2–3.

4.2.1.2.4 Oestrogen receptor antagonism

The T47D-KB*luc* cell line was also used to assess anti-oestrogenic effects of samples (1, 3, 9, 28, 83, and 250 mg/mL). During the bioassay, the cells received a background concentration of E_2 (5.4 pg/mL) ng/mL) which caused 80% activation of the ER during a previous assay (results not shown). For ER inhibition the reference compound was ICI and sample responses were expressed in terms of %ICI Max. The concentration-response curve for ICI (**Figure 25**) was used to determine the following inhibitory concentrations: 0.06 ng/mL (IC_{20}), 0.22 ng/mL (IC_{50}), and 0.85 ng/mL (IC_{80}).

Compared to the SC, very similar effects on the cell viability of the T47D-KB*luc* cell line were seen for the MP (64–144%) and VH (64–147%) samples (**Table 13**). None of the samples caused cytotoxicity with the cell viability of most of the concentrations > 80%. Five samples significantly increased ($p \leq 0.05$) cell viability above 100%: M3 (28 mg/mL; 128%), M5 (250 mg/mL; 133%), M13 (83 mg/mL; 123%), M15 (28 mg/mL; 121% and 250 mg/mL; 132%), and P4 (28 mg/mL; 122%). Since the cell viability was 99% for BC, the increase in viability was not because of the background of E_2 the cells received. These effects could be attributed to other unknown water-soluble compounds present in the samples.

Sample responses for anti-oestrogenic activity were expressed in terms of the reference compound as %ICI Max. As none of the samples caused cytotoxicity in the T47D-KB*luc* cells, low %ICI values can be attributed to ER antagonism, rather than dead cells (**Table 13**). For MP the %ICI Max ranged from 222–

72% and 236–44% for VH samples. Overall, %ICI Max for MP was > 80%, indicating no inhibitory effects on the ER. Similar responses were observed for VH samples. However, there was one exception: M13 showed anti-oestrogenic activity. Nonetheless, %ICI Max values for M13 did not resemble a classical concentration response curve (as shown in **Figure 25**). Consequently, FC could only be calculated for 83 mg/mL of M13 which corresponds to the lowest %ICI Max (44%) (**Figure 26**). Since the FC for M13 was < 1 ($p \leq 0.05$), it confirms ER inhibition. The %ICI Max for the SC was 109%, indicating that the ethanol used to prepare the reference compound concentration range did not affect the outcome of the bioassay.

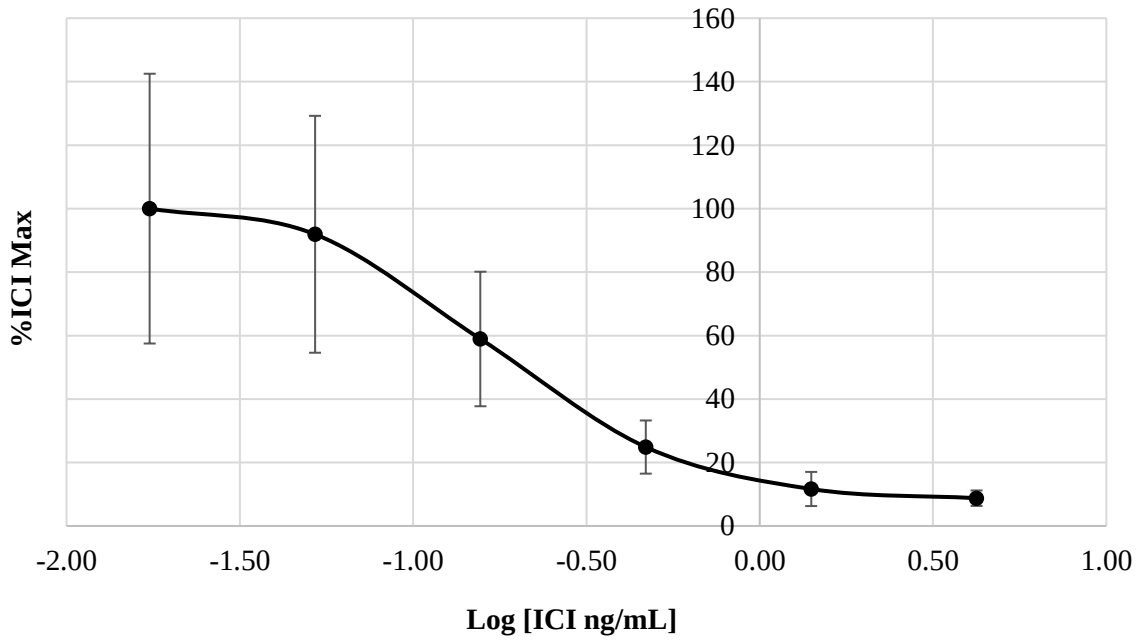

Figure 25 The concentration-response curve obtained for the oestrogen receptor (ER) antagonist reference compound, ICI, during the T47D-KB*luc* bioassay. These cells received a background of the ER agonist, 17β-oestradiol (5.4 pg/mL), to ensure 80% activation of the ER. Luciferase activity is expressed as %ICI Max against the logarithmically transformed ICI exposure concentrations (0.02, 0.05, 0.16, 0.47, 1.41, and 4.22 ng/mL). Error bars indicate the standard deviation.

Table 13 %ICI Max and cell viability (%) values of the T47D-KB*luc* cells after exposure to the samples during the assessment of ER antagonism

Sample name	Exposure concentration (mg/mL)	%ICI Max	Cell viability (%)
Controls			
BC	N/A	103 ± 32.37	99 ± 18.85
NC	N/A	-	*0 ± 1.63
SC	N/A	109 ± 39.93	100 ± 13.58
Mpumalanga province sampling locations			

Sample name	Exposure concentration (mg/mL)	%ICI Max	Cell viability (%)
M1	1	136 ± 4.31	-
	3	176 ± 3.72	-
	9	112 ± 23.50	-
	28	118 ± 11.06	64 ± 7.37
	83	144 ± 41.45	82 ± 18.26
	250	161 ± 47.58	78 ± 36.86
M2	1	194 ± 8.36	-
	3	99 ± 10.78	-
	9	94 ± 8.30	-
	28	92 ± 11.70	77 ± 26.48
	83	118 ± 17.36	90 ± 19.60
	250	*212 ± 21.10	100 ± 21.36
M3	1	121 ± 8.03	-
	3	114 ± 33.87	-
	9	222 ± 18.55	-
	28	100 ± 30.75	*128 ± 8.76
	83	94 ± 18.49	104 ± 10.26
	250	*137 ± 9.23	98 ± 29.59
M4	1	90 ± 6.47	-
	3	123 ± 18.11	-
	9	111 ± 39.51	-
	28	73 ± 1.49	106 ± 24.65
	83	72 ± 10.25	99 ± 10.07
	250	*198 ± 27.38	101 ± 18.62
M5	1	126 ± 13.39	-
	3	113 ± 2.55	-
	9	96 ± 6.86	-
	28	140 ± 10.26	102 ± 13.79
	83	100 ± 3.88	107 ± 4.74
	250	141 ± 39.39	*133 ± 9.88
M6	1	*141 ± 8.95	-
	3	122 ± 2.26	-
	9	146 ± 42.41	-
	28	122 ± 16.83	84 ± 12.52
	83	127 ± 3.71	110 ± 14.74
	250	*186 ± 32.10	97 ± 6.62
M7	1	89 ± 6.76	-
	3	118 ± 13.94	-
	9	126 ± 22.39	-
	28	106 ± 11.74	98 ± 14.17
	83	105 ± 17.90	124 ± 22.63
	250	*169 ± 25.23	85 ± 20.34
M8	1	99 ± 9.26	-

Sample name	Exposure concentration (mg/mL)	%ICI Max	Cell viability (%)
	3	120 ± 18.42	-
	9	94 ± 5.25	-
	28	113 ± 25.17	86 ± 15.47
	83	107 ± 11.83	129 ± 21.78
	250	100 ± 30.14	129 ± 21.86
M9	1	84 ± 14.15	-
	3	105 ± 27.32	-
	9	100 ± 26.32	-
	28	104 ± 15.63	108 ± 25.41
	83	114 ± 28.83	107 ± 3.47
	250	109 ± 26.13	146 ± 34.74
M10	1	105 ± 2.49	-
	3	96 ± 0.38	-
	9	99 ± 4.57	-
	28	101 ± 4.40	97 ± 24.87
	83	108 ± 2.87	122 ± 25.41
	250	*143 ± 13.86	144 ± 37.15
M11	1	107 ± 14.65	-
	3	94 ± 3.68	-
	9	91 ± 10.52	-
	28	97 ± 7.41	88 ± 9.64
	83	95 ± 13.33	103 ± 10.04
	250	126 ± 25.10	83 ± 19.67
Vaalharts Valley, Northern Cape province sampling locations			
M12	1	91 ± 24.99	-
	3	102 ± 6.25	-
	9	85 ± 6.87	-
	28	96 ± 25.20	90 ± 25.81
	83	114 ± 11.99	93 ± 17.78
	250	106 ± 19.21	*73 ± 13.80
M13	1	*61 ± 6.38	-
	3	*5 ± 10.57 7	-
	9	75 ± 36.97	-
	28	*51 ± 10.59	99 ± 15.23
	83	*44 ± 13.66	*123 ± 17.55
	250	*58 ± 14.30	113 ± 17.15
M14	1	53 ± 4.26	-
	3	*57 ± 12.20	-
	9	*51 ± 6.34	-
	28	*57 ± 4.41	113 ± 5.60
	83	*63 ± 7.63	114 ± 7.38
	250	114 ± 35.78	112 ± 17.41

Sample name	Exposure concentration (mg/mL)	%ICI Max	Cell viability (%)
M15	1	117 ± 17.07	-
	3	*166 ± 44.10	-
	9	*212 ± 30.94	-
	28	120 ± 11.27	*121 ± 5.67
	83	148 ± 27.72	133 ± 28.53
	250	*168 ± 13.01	*132 ± 7.53
P1	1	92 ± 20.46	-
	3	124 ± 13.76	-
	9	125 ± 12.08	-
	28	132 ± 4.16	91 ± 16.71
	83	*152 ± 20.86	100 ± 16.18
	250	*146 ± 19.14	99 ± 38.97
P2	1	118 ± 35.69	-
	3	90 ± 13.46	-
	9	*173 ± 31.06	-
	28	147 ± 44.26	90 ± 14.84
	83	133 ± 55.91	64 ± 17.85
	250	236 ± 57.38	88 ± 27.99
P3	1	124 ± 24.56	-
	3	104 ± 15.37	-
	9	127 ± 38.27	-
	28	98 ± 6.20	83 ± 24.59
	83	144 ± 33.31	147 ± 34.89
	250	*225 ± 21.68	112 ± 0.29
P4	1	*58 ± 6.84	-
	3	*56 ± 12.76	-
	9	75 ± 33.02	-
	28	72 ± 15.60	*122 ± 7.00
	83	79 ± 27.46	86 ± 1.19
	250	125 ± 9.27	78 ± 17.00
Non-cytotoxic (≥ 80%)	Weakly cytotoxic (60–79%)	Moderately cytotoxic (41–59%)	Strongly cytotoxic (≤ 40%)

The cytotoxic effect on viability was interpreted according to ISO (2009). All data is presented as mean ± standard deviation. *Statistically significant compared to the SC ($p \leq 0.05$); -cytotoxicity of concentration was not investigated; †cell viability (%) could not be determined due to the presence of bacteria in the wells of the 96-well cell culture test plate; BC: blank control (untreated cells); M1–M15: Maize field 1–Maize field 15; N/A: not applicable; NC: negative control (cells treated with methanol, i.e., non-viable cells); P1–P4: Pecan orchard 1–Pecan orchard 4; SC: solvent control (cells dosed with ethanol, i.e., viable cells).

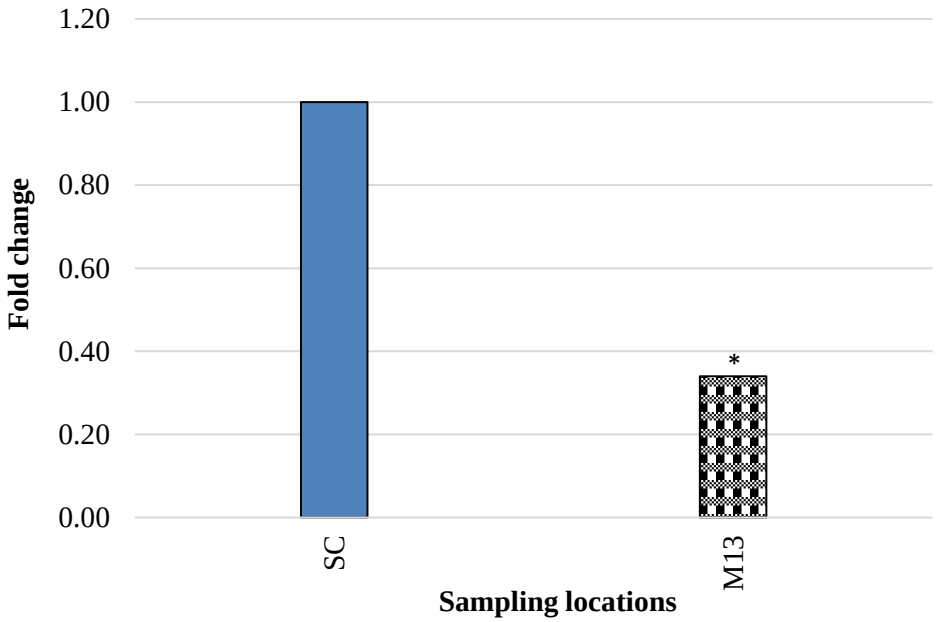

Figure 26 Fold change values calculated for anti-oestrogenic activity of Maize 13 (M13) at 83 mg/mL. Sample responses were compared to the solvent control (SC) (cells dosed with ethanol, i.e., viable cells). *Statistically significantly ($p \leq 0.05$) lower compared to the SC.

4.2.2 Enzymatic and non-enzymatic bioassays

The measurement of enzymatic (SOD, CAT, and AChE) and non-enzymatic (ROS and LPO) endpoints provide an early warning sign for the presence of environmental pollutants, such as pesticides. These endpoints were measured in intestinal (HuTu-80) and liver cells (H4IIE-*luc*) since these organs represent the first line of defence against xenobiotics.

Prior to any enzymatic and non-enzymatic bioassays, an MTT cell viability assay was performed to evaluate the cytotoxicity of the samples towards the HuTu-80 cell line. Cell viability of the HuTu-80 cells ranged from 53–141% for MP samples and from 65–133% for VH samples (**Table 14**). Overall, weak (60–79%) to no cytotoxicity (≥ 80%) towards the HuTu-80 cells was observed after exposure to the different sample concentrations. However, there was an exception for two of the MP samples. Maize 6 and M8 were moderately cytotoxic (41–59%) towards the cells at 250 mg/mL. Yet only the response for M6 was statistically significant ($p \leq 0.05$). For the H4IIE-*luc* cell line, the effect of samples on cell viability was obtained during the assessment of AhR agonism (Section 4.2.1.1.1). Based on the results of the MTT viability assay for both the HuTu-80 (**Table 14**) and H4IIE-*luc* (**Table 9**) cells, 83 mg/mL was overall the highest non-toxic concentration for all samples. Consequently, 83 mg/mL was selected for the assessment of oxidative stress and damage responses, and AChE activity. Data for all enzymatic and non-enzymatic bioassays are presented as mean ± standard deviation, with asterisks indicating statistically significant (*$p \leq 0.05$ and **$p \leq 0.01$) responses compared to the blank control. Cohen's d-value was also calculated

to determine the effect size, with $d \geq 0.8$ indicating practically significant responses. All *p*- and d-values can be found in **Tables S4–S12**.

Table 14 Cell viability (%) of the HuTu-80 cells after exposure to the samples.

Sample name	Exposure concentration (mg/mL)	Cell viability (%)
Controls		
BC	N/A	113 ± 27.96
NC	N/A	*0 ± 2.09
SC	N/A	100 ± 10.27
Mpumalanga province sampling locations		
M1	1	-
	3	-
	9	-
	28	*125 ± 18.66
	83	114 ± 15.40
	250	104 ± 12.80
M2	1	-
	3	-
	9	-
	28	118 ± 13.00
	83	87 ± 2.42
	250	76 ± 19.50
M3	1	-
	3	-
	9	-
	28	97 ± 7.73
	83	77 ± 4.59
	250	107 ± 12.33
M4	1	-
	3	-
	9	-
	28	*78 ± 11.70
	83	*77 ± 14.07
	250	*69 ± 10.90
M5	1	-
	3	-
	9	-
	28	97 ± 12.72
	83	102 ± 18.73
	250	*81 ± 3.56
M6	1	-
	3	-
	9	-
	28	95 ± 9.38

Sample name	Exposure concentration (mg/mL)	Cell viability (%)
	83	94 ± 15.54
	250	*53 ± 9.99
M7	1	-
	3	-
	9	-
	28	101 ± 19.16
	83	79 ± 14.02
	250	*77 ± 4.61
M8	1	-
	3	-
	9	-
	28	*141 ± 1.37
	83	70 ± 4.09
	250	58 ± 6.03
M9	1	-
	3	-
	9	-
	28	115 ± 21.05
	83	*122 ± 11.39
	250	122 ± 21.05
M10	1	-
	3	-
	9	-
	28	*75 ± 7.53
	83	109 ± 13.72
	250	93 ± 6.26
M11	1	-
	3	-
	9	-
	28	92 ± 9.20
	83	111 ± 7.61
	250	81 ± 14.49
Vaalharts Valley, Northern Cape province sampling locations		
M12	1	-
	3	-
	9	-
	28	118 ± 11.86
	83	*133 ± 26.77
	250	107 ± 11.19
M13	1	-
	3	-
	9	-
	28	116 ± 11.98
	83	125 ± 14.76
	250	101 ± 11.80

Sample name	Exposure concentration (mg/mL)	Cell viability (%)
M14	1	-
	3	-
	9	-
	28	*121 ± 1.21
	83	99 ± 12.39
	250	94 ± 22.79
M15	1	-
	3	-
	9	-
	28	95 ± 13.06
	83	97 ± 4.51
	250	94 ± 21.34
P1	1	-
	3	-
	9	-
	28	112 ± 9.38
	83	111 ± 9.15
	250	78 ± 12.02
P2	1	-
	3	-
	9	-
	28	86 ± 4.55
	83	110 ± 5.52
	250	*65 ± 14.13
P3	1	-
	3	-
	9	-
	28	83 ± 9.41
	83	109 ± 20.07
	250	100 ± 19.53
P4	1	-
	3	-
	9	-
	28	106 ± 2.62
	83	111 ± 13.11
	250	95 ± 14.23

The cytotoxic effect on viability was interpreted according to ISO (2009). All data is presented as mean ± standard deviation. *Statistically significant compared to the SC ($p \leq 0.05$); -cytotoxicity of concentration was not investigated; †cell viability (%) could not be determined due to the presence of bacteria in the wells of the 96-well cell culture test plate; BC: blank control (untreated cells); M1–M15: Maize field 1–Maize field 15; N/A: not applicable; NC: negative control (cells treated with methanol, i.e., non-viable cells); P1–P4: Pecan orchard 1–Pecan orchard 4; SC: solvent control (cells dosed with methanol, i.e., viable cells).

4.2.2.1 Oxidative stress and damage

4.2.2.1.1 Intracellular reactive oxygen species production

One source of oxidative stress is ROS. Reactive oxygen species production is part of the normal metabolic processes within cells and the antioxidant defence system is responsible for neutralising excess ROS. However, exposure to xenobiotics such as pesticides can increase intracellular ROS production due to phase I and II biotransformation reactions. Intracellular ROS production was measured using the H_2DCF-DA bioassay. In the presence of ROS, a highly fluorescent derivative is produced, and fluorescence is measured. The amount of fluorescence produced by the sample responses was compared to the fluorescence of the blank control (untreated cells). A PC was also included and was comprised of cells exposed to H_2O_2 which is known to induce ROS production.

HuTu-80 cells stimulated with 3.5 ng/mL of H_2O_2 as the positive control for 45 minutes increased ($d \geq 0.8$) ROS production to 53 617 RFUs compared to the blank control (39 691 RFUs). Exposure to almost half of the MP samples (83 mg/mL) significantly increased ROS levels in HuTu-80 cells above that of the untreated cells: M1, M2, M3, M4, and M5 (**Figure 27** and **Table S4**). The other half of the MP samples significantly reduced the intensity of fluorescence compared to the blank control (untreated cells): M6, M7, M8, M9, M10, and M11 (**Figure 27** and **Table S4**). The elevated and reduced levels of ROS in HuTu-80 cells were also found to be practically significant ($d \geq 0.8$) for these MP samples. Only one VH sample (P1) significantly induced ROS production. Pecan 3 slightly reduced ROS production, although this response was not statistically significant, it was of practical significance. Across all samples, M5 produced the highest amount ROS, producing twice as much fluorescence as the blank control, while M7 was responsible for the biggest reduction ($p \leq 0.01$; $d \geq 0.8$) in ROS (4-fold).

In H4IIE-*luc* cells, the PC (cells stimulated with 14.2 ng/mL hydrogen peroxide for 45 minutes) increased ROS levels compared to the blank control (**Figure 28** and **Table S5**). However, the sample responses were different compared to those in the HuTu-80 cells (**Figure 27** and **Table S4**). Three MP samples significantly increased the intensity of fluorescence: M1, M5, and M7. On the other hand, three other MP samples slightly reduced ROS levels: M4, M8 and M9. A similar trend was seen for the VH samples as with the MP samples: M12, M13 and M14 induced ROS, while M15, P3 and P4 caused a reduction in ROS production.

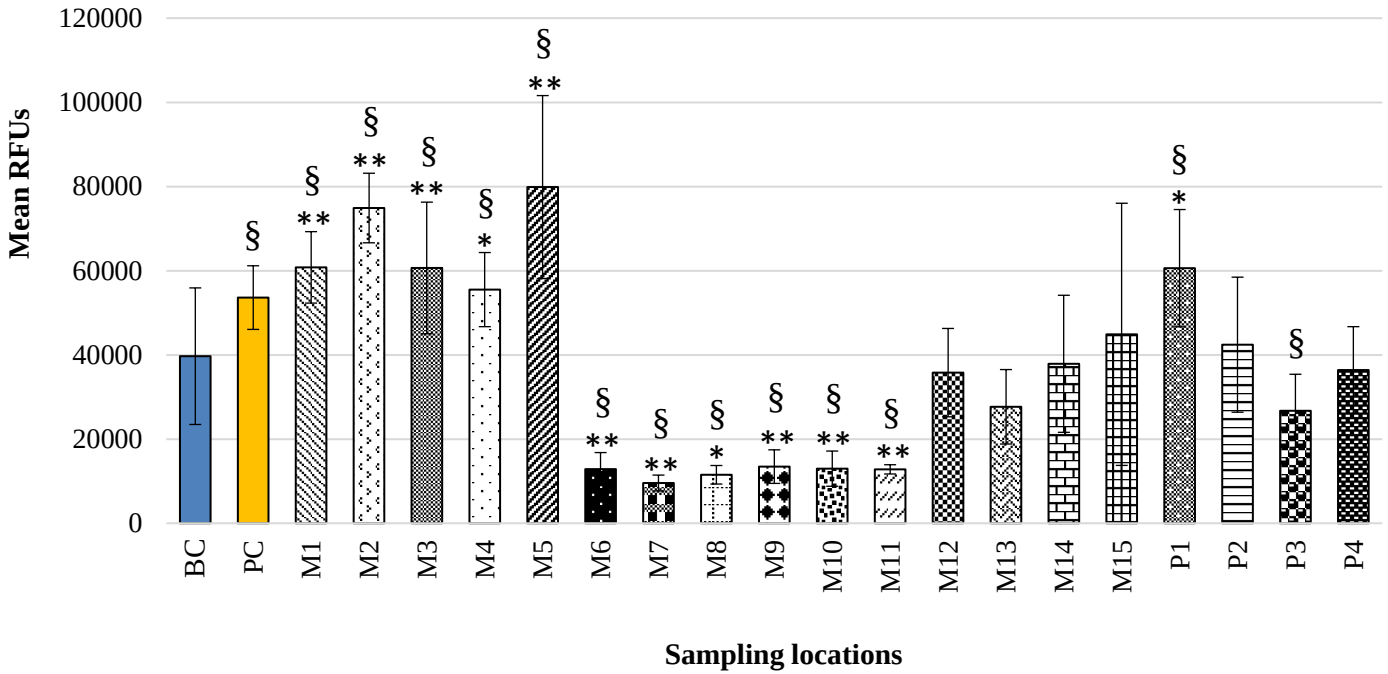

Figure 27 Reactive oxygen species production in HuTu-80 cell line exposed to the samples (83 mg/mL) for 24 hours. Statistically significant differences compared to the blank control (BC; untreated cells) are indicated by asterisks (*$p \leq 0.05$ and **$p \leq 0.01$). § Indicates practically significant differences ($d \geq 0.8$) compared to BC. M1–M15: Maize field 1–Maize field 15; P1–P4: Pecan orchard 1–Pecan orchard 4; PC: Positive control (cells stimulated with 3.5 ng/mL of hydrogen peroxide for 45 minutes); RFUs: Relative fluorescence units. Error bars indicate the standard deviation.

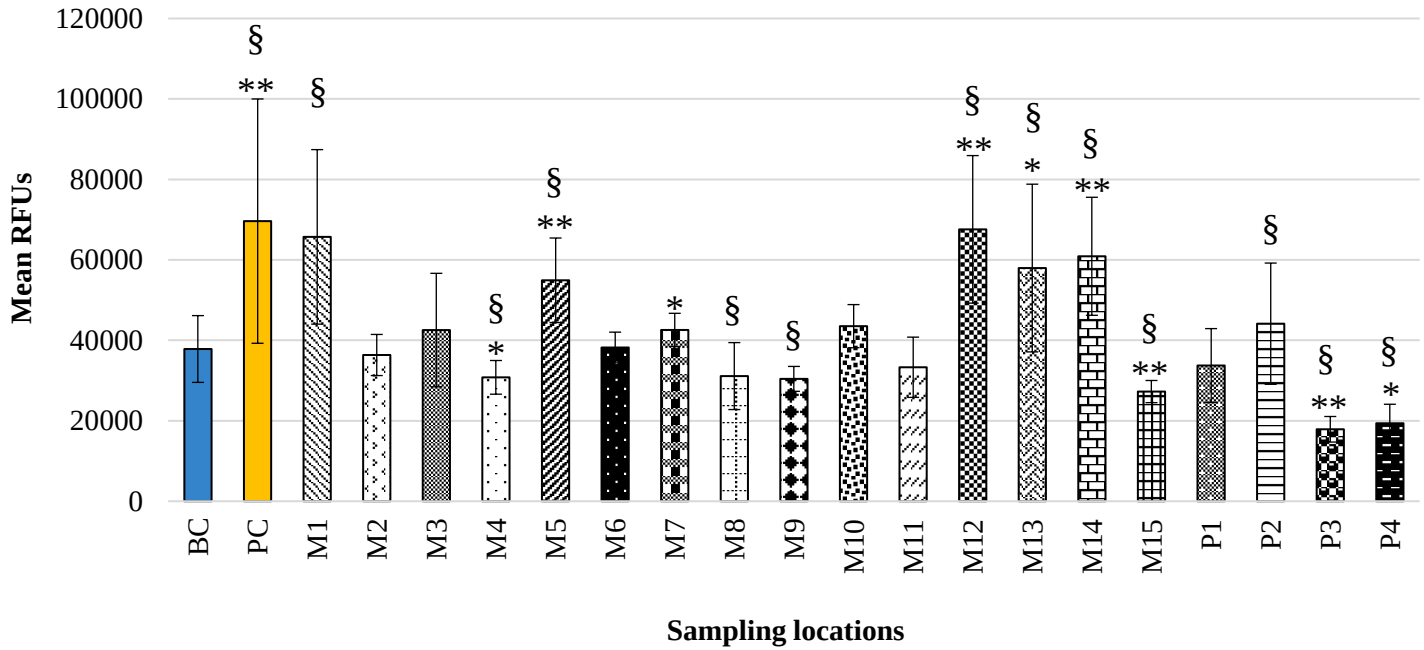

Figure 28 Reactive oxygen species production in H4IIE-*luc* cell line exposed to the samples (83 mg/mL) for 24 hours. Statistically significant differences compared the blank control (BC; untreated cells) are indicated by asterisks (*$p \leq 0.05$ and **$p \leq 0.01$). § Indicates practically significant differences ($d \geq 0.8$) compared to BC. M1–M15: Maize field 1–Maize field 15; P1–P4: Pecan orchard 1–Pecan orchard 4; PC: Positive control (cells stimulated with 14.2 ng/mL of hydrogen peroxide for 45 minutes); RFUs: Relative fluorescence units. Error bars indicate the standard deviation.

4.2.2.1.2 Superoxide dismutase content

Superoxide dismutase is responsible for the conversion of superoxide radicals into hydrogen peroxide and oxygen. Superoxide dismutase content was determined in HuTu-80 and H4IIE-*luc* cells after exposure to the samples (83 mg/mL) by measuring the rate of inhibition of pyrogallol autoxidation. As illustrated in **Figure 29** and **Table S6**, five MP samples increased SOD content over the blank control (untreated cells) in HuTu-80 cells: M1, M3, M7, M10, and M11. Only one MP sample, M8, significantly reduced ($p \leq 0.01$; $d \geq 0.8$) SOD content to10 ng SOD/mg protein. All the VH samples significantly inhibited SOD activity in the HuTu-80 cells: M12, M13, M14, P1, P2, P3, and P4 (**Figure 29** and **Table S6**).

Exposure to MP samples caused an increase in SOD content in the H4IIE-*luc* cells (**Figure 30** and **Table S7**). This included M2, M4, M5, M6, M7, and M8. Yet, there was one exception: exposure to 83 mg/mL of M11 significantly reduced ($p \leq 0.05$; $d \geq 0.8$) SOD content to 40.29 ng SOD/mg protein. No significant responses (increase or decrease) for SOD content were observed when the H4IIE-*luc* cells were exposed to the VH samples.

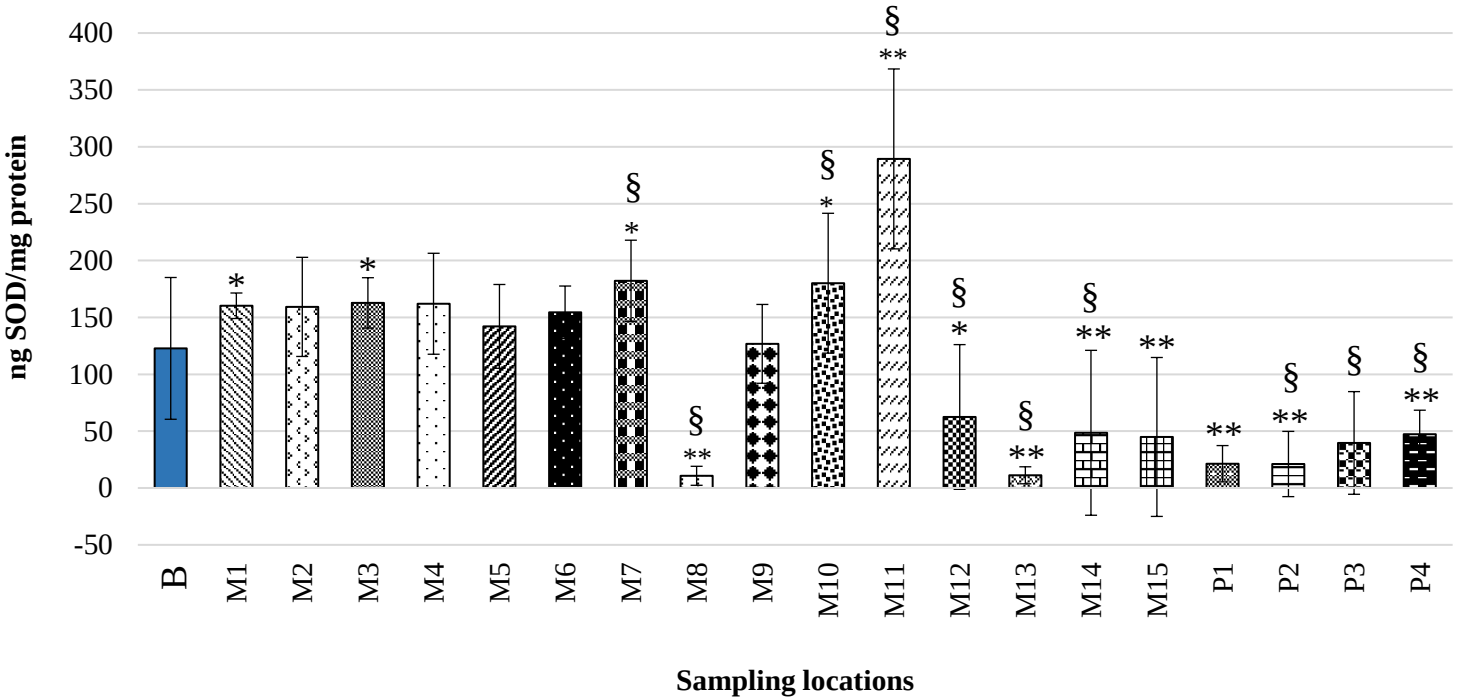

Figure 29 Superoxide dismutase content in HuTu-80 cell line exposed to the samples (83 mg/mL) for 24 hours. Statistically significant differences compared the blank control (BC; untreated cells) are indicated by asterisks (*$p \leq$ 0.05 and **$p \leq 0.01$). § Indicates practically significant differences ($d \geq 0.8$) compared to BC; M1–M15: Maize field 1–Maize field 15; P1–P4: Pecan orchard 1–Pecan orchard 4; SOD: superoxide dismutase. Error bars indicate the standard deviation.

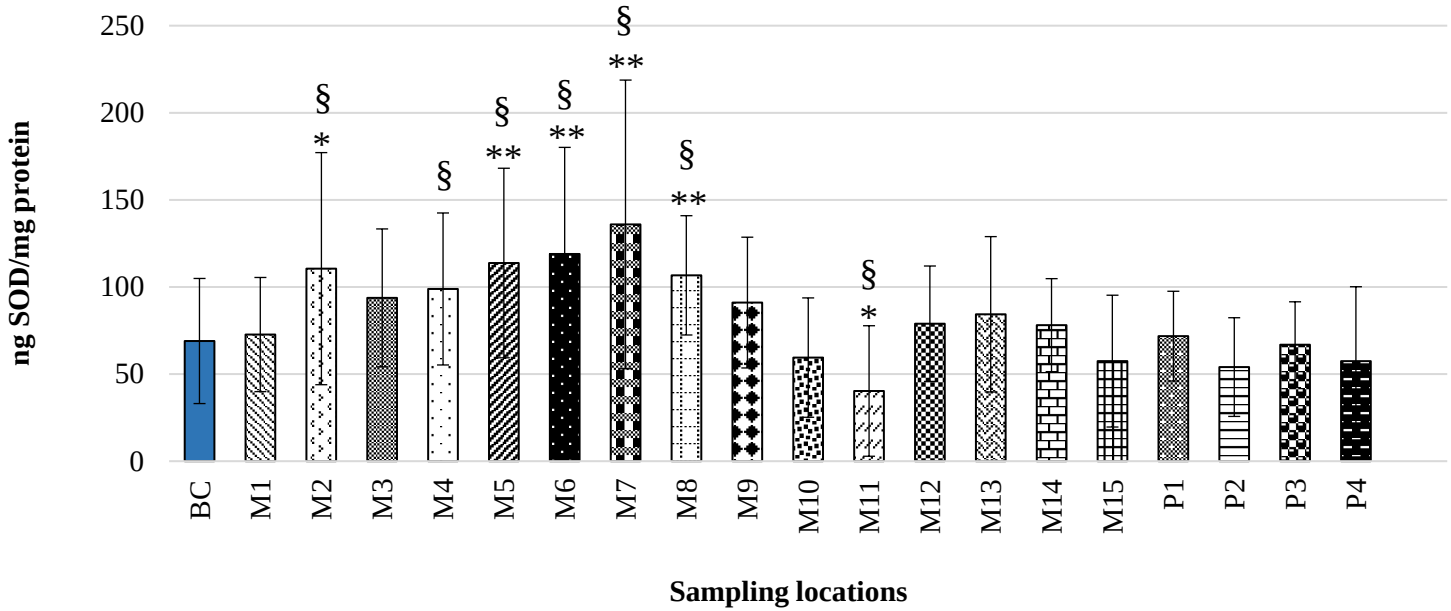

Figure 30 Superoxide dismutase content in H4IIE-*luc* cell line exposed to the samples (83 mg/mL) for 24 hours. Statistically significant differences compared the blank control (BC; untreated cells) are indicated by asterisks (*$p \leq 0.05$ and **$p \leq 0.01$). § Indicates practically significant differences ($d \geq 0.8$) compared to BC; M1–M15: Maize field 1–Maize field 15; P1–P4: Pecan orchard 1–Pecan orchard 4; SOD: superoxide dismutase. Error bars indicate the standard deviation.

4.2.2.1.3 Catalase activity

Together with SOD, CAT forms the first-line of defence against ROS. Catalase converts the reactive hydrogen peroxide produced during SOD detoxification actions into molecular oxygen and water. After the HuTu-80 and H4IIE-*luc* cells were exposed to the samples (83 mg/mL) for 24 hours, CAT activity was measured by means of titration of KMnO4, a very strong oxidising reagent that reacts with H_2O_2.

The results in **Figure 31** and **Table S8** show that in general the MP samples reduced CAT activity in the HuTu-80 cells. However, most of these responses were not statistically or practically significant. The lowest CAT activity was observed after exposure to M9 which significantly reduced (12.13 µM H_2O_2/min/mg protein; $p \leq 0.05$) enzyme activity compared to the untreated blank control (27.87 µM H_2O_2/min/mg protein). Four of the MP samples significantly increased CAT activity: M7, M8, M10, and M11. Exposure to all the VH samples increased CAT activity with the majority of the responses of statistical and practical significance: M12, M13, M14, M15, P2, P3, and P4 (**Figure 31** and **Table S8**).

For the CAT activity in the H4IIE-*luc* cells, the majority of the MP samples did not cause any change in CAT activity compared to the blank control (42.07 µM H_2O_2/min/mg protein) (**Figure 32** and **Table S9**). Only one of the MP samples showed any significant response: exposure to M9 increased CAT activity to 73.52 µM H_2O_2/min/mg protein ($p \leq 0.01$; $d \geq 0.8$). Overall, CAT activity in cells exposed to the VH sample also significantly increased for M12, M14, P1, and P3 (**Figure 32** and **Table S9**).

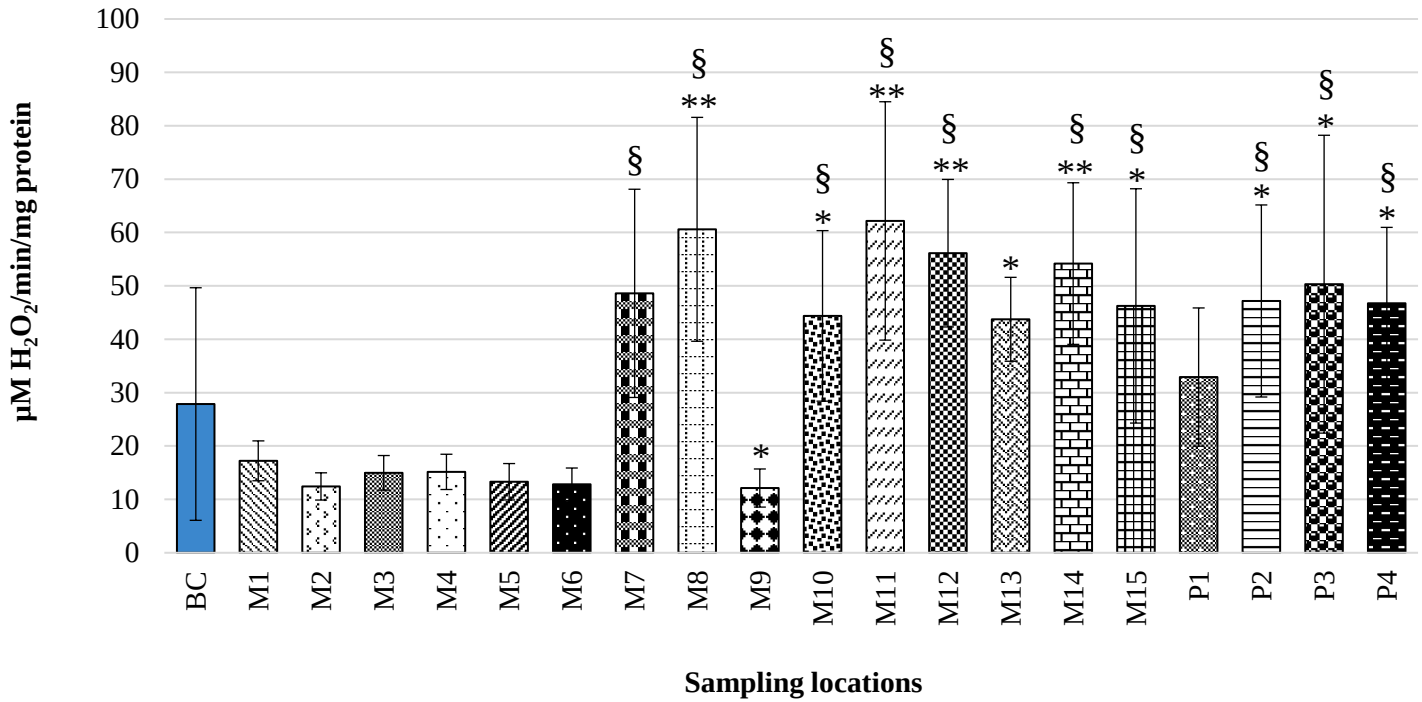

Figure 31 Catalase activity in HuTu-80 cell line exposed to the samples (83 mg/mL) for 24 hours. Statistically significant differences compared to the blank control (BC; untreated cells) are indicated by asterisks (*$p \leq 0.05$ and **$p \leq 0.01$). § Indicates practically significant differences ($d \geq 0.8$) compared to BC; H_2O_2: hydrogen peroxide; M1–M15: Maize field 1–Maize field 15; P1–P4: Pecan orchard 1–Pecan orchard 4. Error bars indicate the standard deviation.

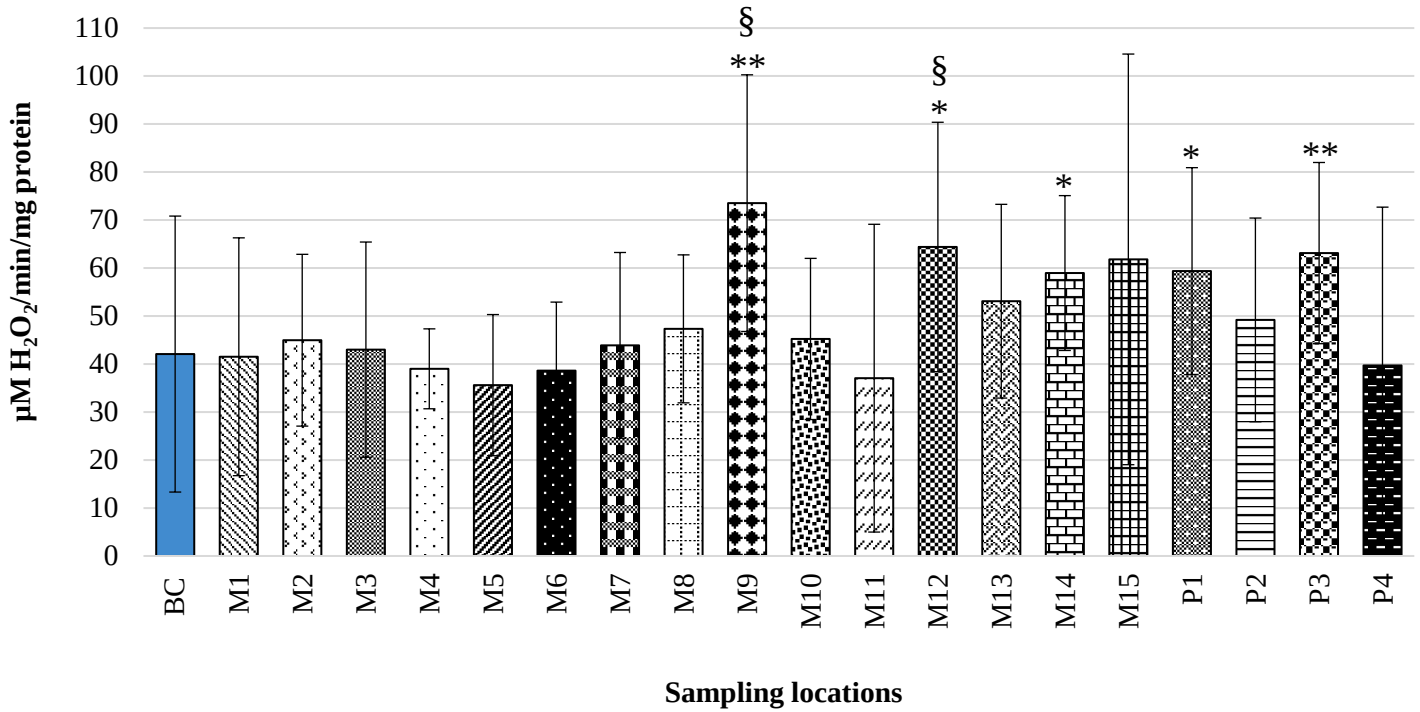

Figure 32 Catalase activity in H4IIE-*luc* cell line exposed to the samples (83 mg/mL) for 24 hours. Statistically significant differences compared to the blank control (BC; untreated cells) are indicated by asterisks (*$p \leq 0.05$ and **$p \leq 0.01$). § Indicates practically significant differences ($d \geq 0.8$) compared to BC; H_2O_2: hydrogen peroxide; M1–M15: Maize field 1–Maize field 15; P1–P4: Pecan orchard 1–Pecan orchard 4. Error bars indicate the standard deviation.

4.2.2.1.4 Lipid peroxidation as malondialdehyde content

When the antioxidant defence system can no longer mitigate the effects of oxidative stress, it leads to oxidative damage, such as the oxidative degradation of lipid components within cellular membranes (LPO). The TBARS assay was used to determine LPO by measuring the oxidative end-product, MDA, in cells exposed to the samples (83 mg/mL) for 24 hours. The intracellular MDA content (mM MDA/mg protein) in HuTu-80 (**Figure 33** and **Table S10**) and H4IIE-*luc* (**Figure 34** and **Table S11**) cells after exposure to the samples could not be calculated from the MDA calibration curve. Very low absorbance values were obtained compared to the TMP standard, indicating that no LPO occurred in either of the cell lines after 24 hours of exposure to the samples. However, there were statistically and practically significant differences between the response of all the TMP concentrations (60, 90, 120, 150, 180, 210, and 240 mM) and the blank control (untreated cells). Since absorbance values for the TMP standard increased ($p \leq 0.1$; $d \geq 0.8$) in a concentration-dependent manner (**Figure 33** and **Figure 34**), it indicates that the experimental procedure followed for the TBARS assay worked and TMP was converted into MDA under conditions of high temperature and acidity.

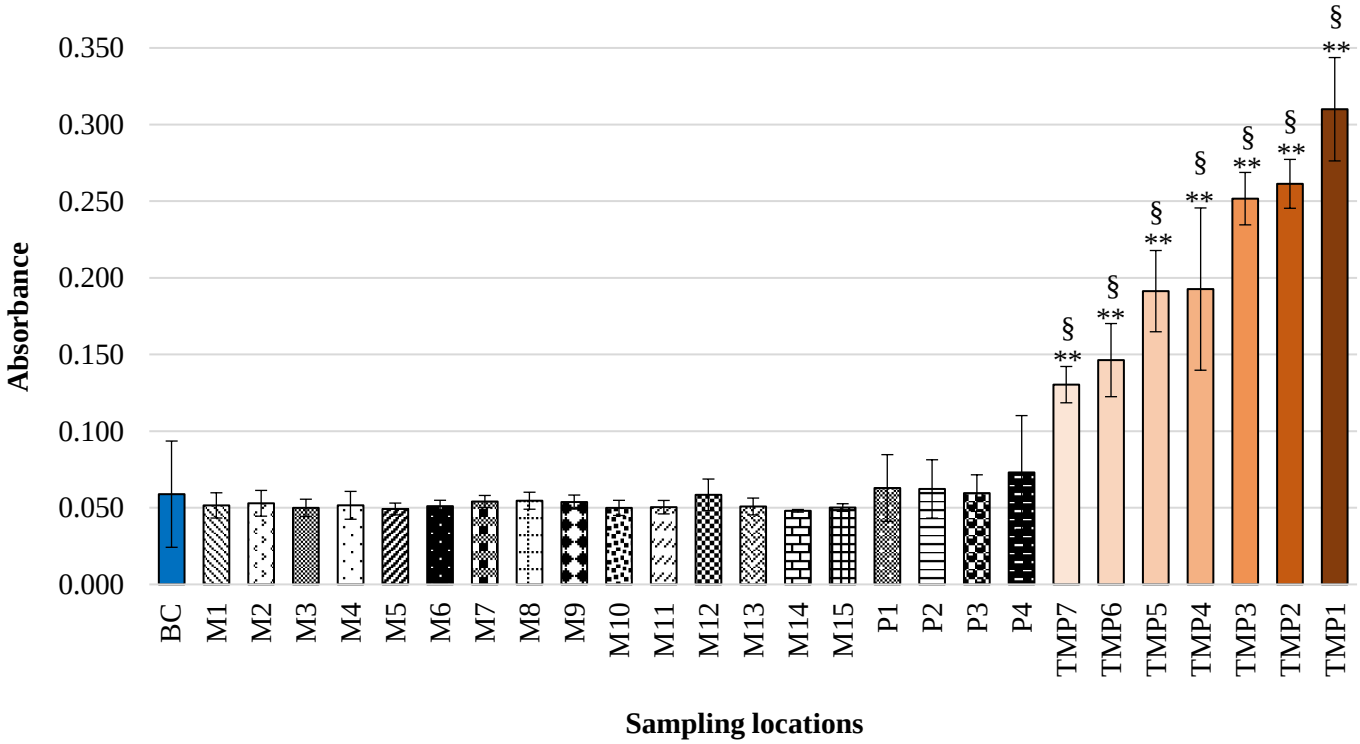

Figure 33 Lipid peroxidation as malondialdehyde content in HuTu-80 cell line exposed to the samples (83 mg/mL) for 24 hours. Statistically significant differences compared to the blank control (BC; untreated cells) are indicated by asterisks (*$p \leq 0.05$ and **$p \leq 0.01$). § Indicates practically significant differences ($d \geq 0.8$) compared to BC; M1–M15: Maize field 1–Maize field 15; P1–P4: Pecan orchard 1–Pecan orchard 4; TMP: 1,1,3,3-tetramethoxypropane. Error bars indicate the standard deviation.

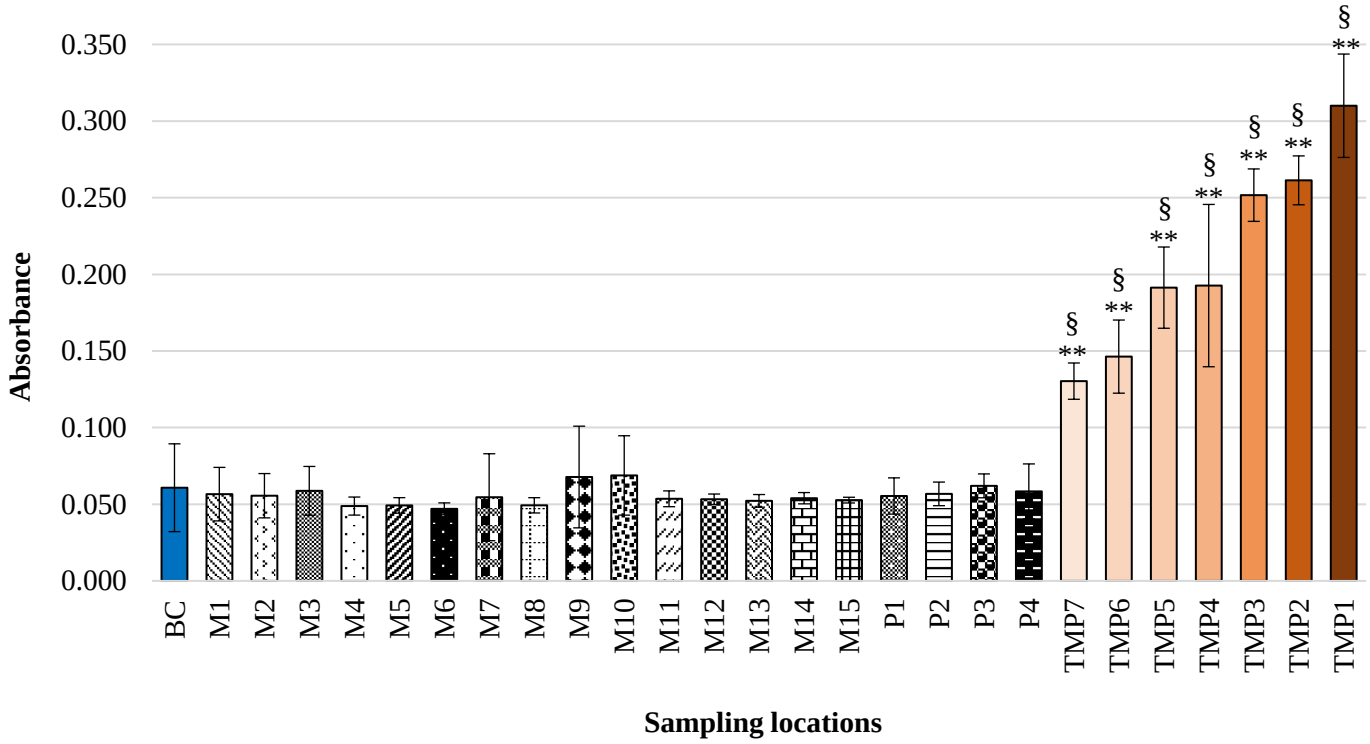

Figure 34 Lipid peroxidation as malondialdehyde content in H4IIE-*luc* cell line exposed to the samples (83 mg/mL) for 24 hours. Statistically significant differences compared to the blank control (BC; untreated cells) are indicated by asterisks (*$p \leq 0.05$ and **$p \leq 0.01$). § Indicates practically significant differences ($d \geq 0.8$) compared to BC; M1–M15: Maize field 1–Maize field 15; P1–P4: Pecan orchard 1–Pecan orchard 4; TMP: 1,1,3,3-tetramethoxypropane. Error bars indicate the standard deviation.

4.2.2.1.5 Acetylcholinesterase activity

Acetylcholinesterase is responsible for the hydrolysis of ACh to choline and acetate. Although AChE is primarily found at neuromuscular junctions and cholinergic synapses in the central nervous system, it is also produced in the intestines and liver as part of the NNCS. These organs play an important role in the detoxification of xenobiotics, including pesticides. AChE inhibition in intestinal and liver cells causes damage to non-neuronal cholinergic transmission in important detoxification organs. After exposure to the samples (83 mg/mL) for 24 hours, AChE activity in the HuTu-80 and H4IIE-*luc* cells was determined using the Ellman assay which had been adapted for measurement in a 96-well microplate. The HuTu-80 cells did not produce any detectable amounts of AChE (results not shown). On the other hand, the AChE activity in the H4IIE-*luc* cells is shown in **Figure 35** and **Table S12**. The majority of the MP and VH samples caused a marked decrease in AChE activity compared to the blank control (4.78×10^{-4} absorbance/min/mg protein). However, overall, these responses were not statistically or practically significant when compared to the untreated control (BC). Only four samples showed statistically significant AChE inhibition: M10, M12, M13, and M14. On the other hand, M7 increased AChE activity in the H4IIE-*luc* cells 1.5-fold (7.50×10^{-4} absorbance/min/mg protein; $p \leq 0.01$) compared to the untreated control (BC).

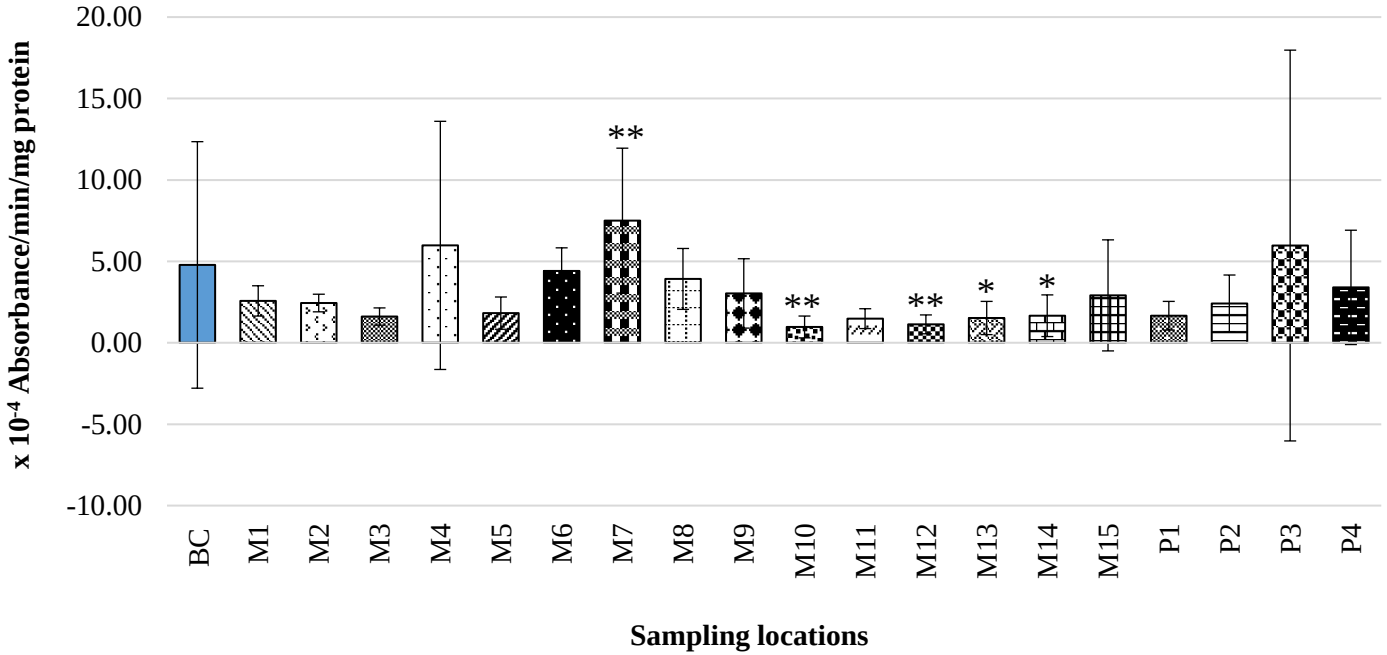

Figure 35 Acetylcholinesterase activity in H4IIE-*luc* cell line exposed to the samples (83 mg/mL) for 24 hours. Statistically significant differences compared to the blank control (BC; untreated cells) are indicated by asterisks (*$p \leq 0.05$ and **$p \leq 0.01$). § Indicates practically significant differences ($d \geq 0.8$) compared to BC; M1–M15: Maize field 1–Maize field 15; P1–P4: Pecan orchard 1–Pecan orchard 4. Error bars indicate the standard deviation.

4.3 Instrumental chemical analysis

4.3.1 Method validation

The analytical method developed in this study was applied to measure the concentrations of four pesticides in soil from maize fields and pecan orchards in South Africa. The LODs and LOQs of 2,4-D, atrazine, dicamba and imidacloprid for the method followed in this study are reported in **Table 15**. The standard curves for each of the four pesticides had correlation coefficients of ≥ 0.995. According to the results obtained for the validation method parameters the linearity, precision and accuracy were acceptable (**Table 15**). The results obtained for the laboratory blanks were all < LOQ, confirming that the sample processing steps did not introduce any pesticides. Mean recoveries of all four pesticides in the QC samples were below 50%, with atrazine (42%) and dicamba (22%) having the highest and lowest recoveries, respectively (**Table 15**). The calibration curve ranges established in this study using UHPLC-QTOF/MS for 2,4-D, atrazine, dicamba and imidacloprid were 0.0–2.0, 0.0–0.1, 0.0–10.5, and 0.0–10.0 µg/mL, respectively (**Table 15**).

Accelerated solvent extraction was used to extract the four target pesticides from the soil samples. Overall, low recoveries were obtained in this study (**Table 15**). For 2,4-D and atrazine the recoveries were comparable to those reported previously for polar pesticides from soil. The 36% recovery of 2,4-D was in the range of the recoveries (25–67%) for 2,4-D during a field study conducted by Voos *et al*. (1994). The recovery obtained in this study for atrazine (42%) was comparable to findings by Guzzella and Pozzoni (1999) who reported 47% recovery after spiking soil with 10 ng/g atrazine. Although both are synthetic auxin herbicides, dicamba showed considerably lower recovery (22%) from the soil compared to 2,4-D. This was in contrast with the findings of Voos *et al*. (1994) who reported higher recovery for dicamba (65–137%) than for 2,4-D (25–67%). During this study, only 32% recovery was found for imidacloprid (**Table 15**). However, these results were not comparable with current literature as most studies showed higher recoveries for imidacloprid in soil: 77.1–94.8% (Bonmatin *et al*., 2003); 82.6–109% (Moghaddam *et al*., 2012); and 78.8% (Schaafsma *et al*., 2015). This was especially the case when QuEChERS (quick, easy, cheap, effective, rugged, and safe) extraction kits were used for extractions, as opposed to ASE. According to Guzzella and Pozzoni (1999) the recovery (%) compounds in the of topsoil (0–25 cm) samples is usually lower compared to deeper soil samples due to the complexity of the matrix (i.e., the presence of humic acids, water, and other organic pollutants). This could explain the recoveries below 50% for 2,4-D, atrazine and imidacloprid in the soil samples. Although a matrix-matched calibration curve was used to account for matrix effects, the recoveries were still low as the extraction method was not optimised for each target compound, but the aim was rather to obtain a mixture of agrochemicals by using one extraction method. Additionally, the ASE parameters could have influenced the extraction process. Although 60°C was used during this study, Kremer *et al*. (2004) demonstrated that an extraction temperature of 100°C showed better recovery for acidic pesticides, such as 2,4-D.

4.3.1 Quantification of target pesticides in soil samples

Table 16 shows that all four target pesticides (2,4-D, atrazine, dicamba and imidacloprid) were detected at quantifiable concentrations in the soil samples. Pesticide concentrations ranged from 4.5–832.4 ng/g for 2,4-D, 0.2–208.6 ng/g for atrazine, 49.4–8 1050.0 ng/g for dicamba, and 19.5–96.9 ng/g for imidacloprid. The detection frequencies in the samples were as follows: atrazine (89%) > dicamba (84%) > 2,4-D (74%) > imidacloprid (32%) (**Table 16**). The highest pesticide concentration was quantified in M11 (dicamba; 8 1050.0 ng/g, and the lowest in P4 (atrazine; 0.2 ng/g). Four of the samples had quantifiable amounts of all four pesticides: M1, M4, M14, and M15. Overall, M11 had the highest pesticide load (ΣPesticides = 8 108.9 ng/g), followed by M14 (ΣPesticides = 1 692.4 ng/g) and M6 (ΣPesticides = 1 465.0 ng/g). Maize 12 had the lowest pesticide load (ΣPesticides = 9.4 ng/g) (**Table 16**). These findings show that selected pesticides are present in the bioavailable fraction of agricultural soils in South Africa.

Table 15 Results of quality control and quality assurance.

Target compound	Calibration curve concentration range	Linearity	LOD	LOQ	Inter-day precision (%RSD)			Intra-day precision (%RSD)	Accuracy (%)
	µg/mL	R2	µg/mL	µg/mL	Day 1	Day 2	Day 3	<15%	(Recovery)
2,4-D	0.0, 0.25, 0.5, 1.3, 2.0	0.995	0.197	0.655					36.41
Low					8.73	6.25	8.41	10.52	
High					2.56	2.24	5.75	5.17	
Medium					5.76	2.24	3.26	5.17	
Atrazine	0.0, 0.005, 0.0125, 0.05, 0.1	0.999	0.005	0.016					42.38
Low					10.62	18.37	12.70	20.22	
Medium					7.71	7.22	6.45	12.89	
High					3.26	5.50	8.34	14.70	
Dicamba	0.0, 2.0, 3.8, 6.0, 10.5	0.999	0.258	0.861					22.08
Low					22.17	13.40	22.68	24.00	
Medium					17.85	14.92	14.32	18.21	
High					21.37	9.92	12.08	17.57	
Imidacloprid	0.0, 0.5, 1.2, 6.0, 10.0	0.998	0.622	2.074					31.91
Low					11.62	14.69	3.91	14.60	
Medium					4.97	2.79	6.03	15.67	
High					1.99	3.89	3.39	15.24	

2,4-D: 2,4-Dichlorophenoxyacetic acid; LOD: limit of detection; LOQ: limit of quantification; RSD: relative standard deviation.

Table 16 The concentrations (ng/g) of pesticides in the soil samples.

Sample name	2,4-D	Atrazine	Dicamba	Imidacloprid	ΣPesticides
Mpumalanga province sampling locations					
Maize 1	4.5 ± 4.8	20.9 ± 6.6	65.5 ± 24.2	96.9 ± 34.9	187.8 ± 70.5
Maize 2	747.3 ± 216.6	43.4 ± 9.8	49.4 ± 17.9	< LOQ	840.1 ± 244.3
Maize 3	553.5 ± 214.3	< LOQ	353.4 ± 338.7	< LOD	906.9 ± 553.0
Maize 4	19.5 ± 3.1	24.6 ± 9.6	127.5 ± 121.5	50.1 ± 14.6	221.7 ± 148.8
Maize 5	< LOQ	4.0 ± 0.9	541.5 ± 311.0	< LOQ	545.5 ± 311.9
Maize 6	832.4 ± 349.8	0.7 ± 0.1	631.9 ± 280.1	< LOD	1 465.0 ± 630.0
Maize 7	< LOD	1.0 ± 0.2	450.0 ± 496.3	< LOQ	451.0 ± 496.5
Maize 8	568.5 ± 753.1	208.6 ± 7.8	< LOD	< LOQ	777.1 ± 760.9
Maize 9	584.0 ± 599.1	8.3 ± 2.3	70.1 ± 122.9	< LOQ	662.4 ± 724.3
Maize 10	< LOQ	13.3 ± 5.6	< LOD	19.5 ± 5.4	32.8 ± 11.0
Maize 11	< LOQ	3.9 ± 5.8	8 105.0 ± 2 597.9	< LOQ	8 108.9 ± 2 603.7
Vaalharts Valley, Northern Cape province sampling locations					
Maize 12	9.4 ± 5.5	< LOQ	< LOQ	< LOD	9.4 ± 5.5
Maize 13	< LOQ	1.8 ± 1.3	280.3 ± 273.7	38.8 ± 27.1	320.9 ± 302.1
Maize 14	688.7 ± 549.3	161.6 ± 85.5	808.2 ± 700.5	33.9 ± 16.9	1 692.4 ± 1 352.2
Maize 15	9.7 ± 16.1	61.9 ± 29.3	209.1 ± 198.4	41.6 ± 20.1	322.3 ± 263.9
Pecan 1	10.4 ± 3.5	0.4 ± 0.4	107.6 ± 187.8	< LOD	118.4 ± 191.7
Pecan 2	22.2 ± 5.8	1.3 ± 0.3	166.5 ± 150.4	< LOQ	190.0 ± 156.5
Pecan 3	16.1 ± 1.9	0.8 ± 0.8	245.0 ± 217.2	< LOD	261.9 ± 219.9
Pecan 4	16.2 ± 4.7	0.2 ± 0.1	175.9 ± 172.7	< LOQ	192.3 ± 177.5

Data are presented as the mean concentrations ± standard deviations; Σ: Sum; 2,4-D: 2,4-Dichlorophenoxyacetic acid; LOD: limit of detection; LOQ: limit of quantification; values in red and blue indicate the highest and lowest concentrations, respectively.

4.3.2 Screening of agrochemicals in soil samples

Following the quantification of 2,4-D, atrazine, diamba, and imidacoprid, the data obtained from the chromatographic analysis was used to screen for the presence of other agrochemicals by matching compound possibilities generated based on molecular features and spectra to Agilent's MassHunter Forensics and Toxicology PCDL. **Figure 36** and **Figure 37** show the number of agrochemicals identified in positive and negative ESI mode, respectively. A total of 2 513 compounds were identified with more for positive (1 470) than negative (1 043) ESI mode. Although fewer compounds were detected overall for negative ESI, M11 was an exception with the second most compounds (160) identified after M2 (positive ESI) with 166 compounds. The least number of compounds (26) were identified in M15 (negative ESI). In general, herbicides (37%) were the most representative classes of agrochemicals identified followed by insecticides (36%), fungicides (17%), other pesticides (5%), acaricides (3%), and plant growth regulators (1%). The identity of the compounds, as well as their identification details (abundance, score, m/z ratio and retention time) are listed in **Table S13** and **Table S14**.

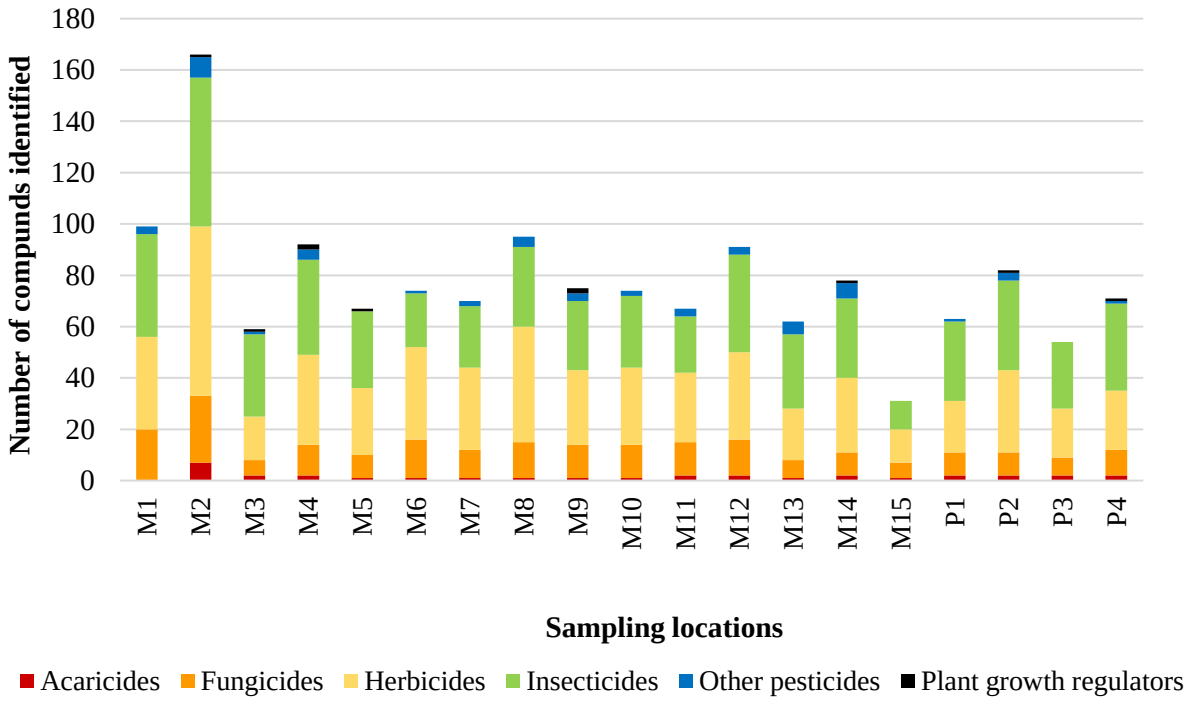

Figure 36 Number of agrochemicals identified in positive electrospray ionisation mode after matching the data obtained from the chromatographic analysis to Agilent's MassHunter Forensics and Toxicology Personal Compound Database and Library; M1–M15: Maize field 1–Maize field 15; P1–P4: Pecan orchard 1–Pecan orchard.

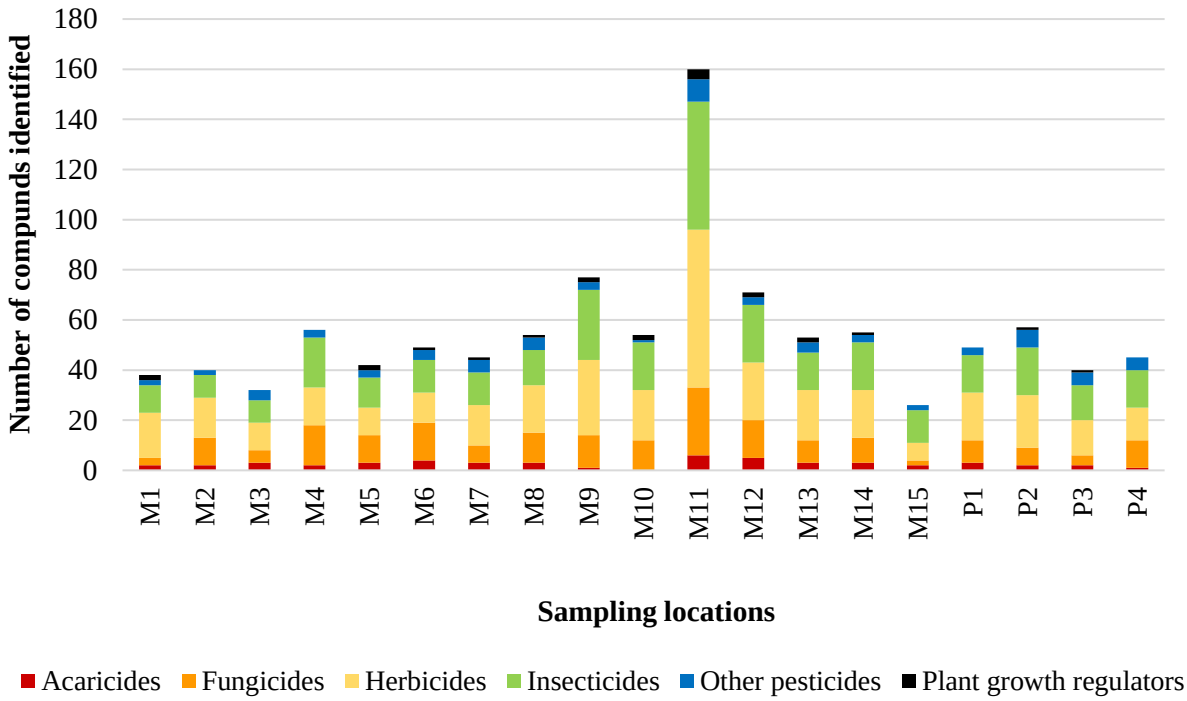

Figure 37 Number of agrochemicals identified in negative electrospray ionisation mode after matching the data obtained from the chromatographic analysis to Agilent's MassHunter Forensics and Toxicology Personal Compound Database and Library; M1–M15: Maize field 1–Maize field 15; P1–P4: Pecan orchard 1–Pecan orchard.

CHAPTER 5: DISCUSSION

Agrochemicals, including pesticides, fertilisers, and synthetic hormones, are extensively used in the South African agriculture industry. However, many of these chemicals are water-soluble and end up in non-target environments following rainfall or irrigation. When bioavailable these chemicals can elicit effects on non-target organisms, posing a threat to ecosystem health. This study investigated the biological effects associated with water-soluble chemicals present in maize and pecan soil from two agricultural regions in South Africa. More specifically, cytotoxicity, xenobiotic metabolism, endocrine disruption, oxidative stress, and damage endpoints were evaluated using a battery of *in vitro* bioassays. This included the use of an MTT cell viability assay for the assessment of non-specific toxicity; reporter gene bioassays that reflect receptor-mediated effects, such as binding to and activating, or blocking and inhibiting nuclear (AhR) and nuclear hormone receptors (AR, GR, and ER); and enzymatic and non-enzymatic bioassays to investigate ROS production, SOD and CAT activity, LPO, and AChE inhibition. Since many pesticides are known to elicit the abovementioned biological effects, four current-use pesticides (2,4-D, atrazine, dicamba, and imidacloprid) were quantified in the soil samples using UHPLC-QTOF/MS (quantitative analysis). The data obtained following UHPLC-QTOF/MS analysis was also compared to a mass spectral library to determine whether other agrochemicals (qualitative analysis) were also present. This chapter presents the interpretation and discussion of the statistically and/or practically significant results reported on in Chapter 4. The results from the soil properties and *in vitro* bioassays have been summarised in **Table 19**. Since studies investigating the biological effects associated with environmental samples, especially soil, are scarce, attention is paid to effects reported in the literature which originated from anthropogenic sources (i.e., pesticide active ingredients and commercial pesticide formulations)

5.1 Soil properties

Since soil can act as both a source and sink of pollutants (Durães *et al*., 2018), physicochemical soil properties such as %TOC and soil particle size distribution can affect the bioavailability of many xenobiotics, including pesticides (Odukkathil & Vasudevan, 2013). Soil organic matter (which includes organic carbon) adsorbs pollutants (Harju *et al*., 2021). Therefore, soils with a high organic content retains pesticides and reduces their mobility through the soil (Navarro *et al*., 2021). In such a case, the chemicals are less likely to leach or run-off to non-target environments and become bioavailable (Harju *et al*., 2021). This was evidenced by Zhang *et al*. (2012) who found that concentrations of the insecticide dichlorodiphenyltrichloroethane (DDT) were positively correlated with TOC, suggesting that these chemicals are more persistent in soils with high TOC as opposed to soils with lower TOC. Soil particle size distribution also plays a role in the release of pollutants into other environments (e.g., waterbodies). Fine particles (< 100 µm) more readily retain pollutants since they present a higher surface higher area (Durães *et al*., 2018). In this study, %TOC and soil particle size distribution of all soil samples were determined.

Overall, all samples had low to moderately low levels of TOC (< 2%) (**Table 7**), which is not uncommon since cultivation practices can decrease the TOC of agricultural soils (Wu *et al*., 2004). A finding from Boyer & Groffman (1996) also showed that agricultural soils have lower total carbon, compared to other soils. It is possible that the low %TOC allow pollutants to migrate from the soil to aquatic environments more readily. Consequently, the relationship between organic carbon content and the biological effects observed in the present study was further investigated. Since bioanalytical EQs could not be calculated for AhR, AR, and ER activity, correlation analysis was only performed for the %TOC and oxidative stress responses.

The correlation between the %TOC and oxidative stress responses of the samples in the HuTu-80 and H4IIE-*luc* cell lines are shown in **Figure 38**. In both the HuTu-80 and H4IIE-*luc* cells the $|r_s|$ of all samples was below 0.6 (**Table 17**), showing only a fair correlation between %TOC and the oxidative stress responses. However, since the $|r_s|$ was not statistically significant for any of the sample responses it can be concluded that the amount of organic carbon did not affect the oxidative stress responses in this study. Similar to these findings, non-significant correlations between both SOD and CAT activity in the amphibious snail *Pila globosa* and soil organic carbon has been shown (Pati *et al*., 2022). On the other hand, Li *et al*. (2003) reported a direct correlation between high organic content in particulate matter and the oxidative stress biomarker, cellular heme oxygenase-I, in murine macrophage (RAW 264.7) and human bronchial epithelial (BEAS-2B) cell lines.

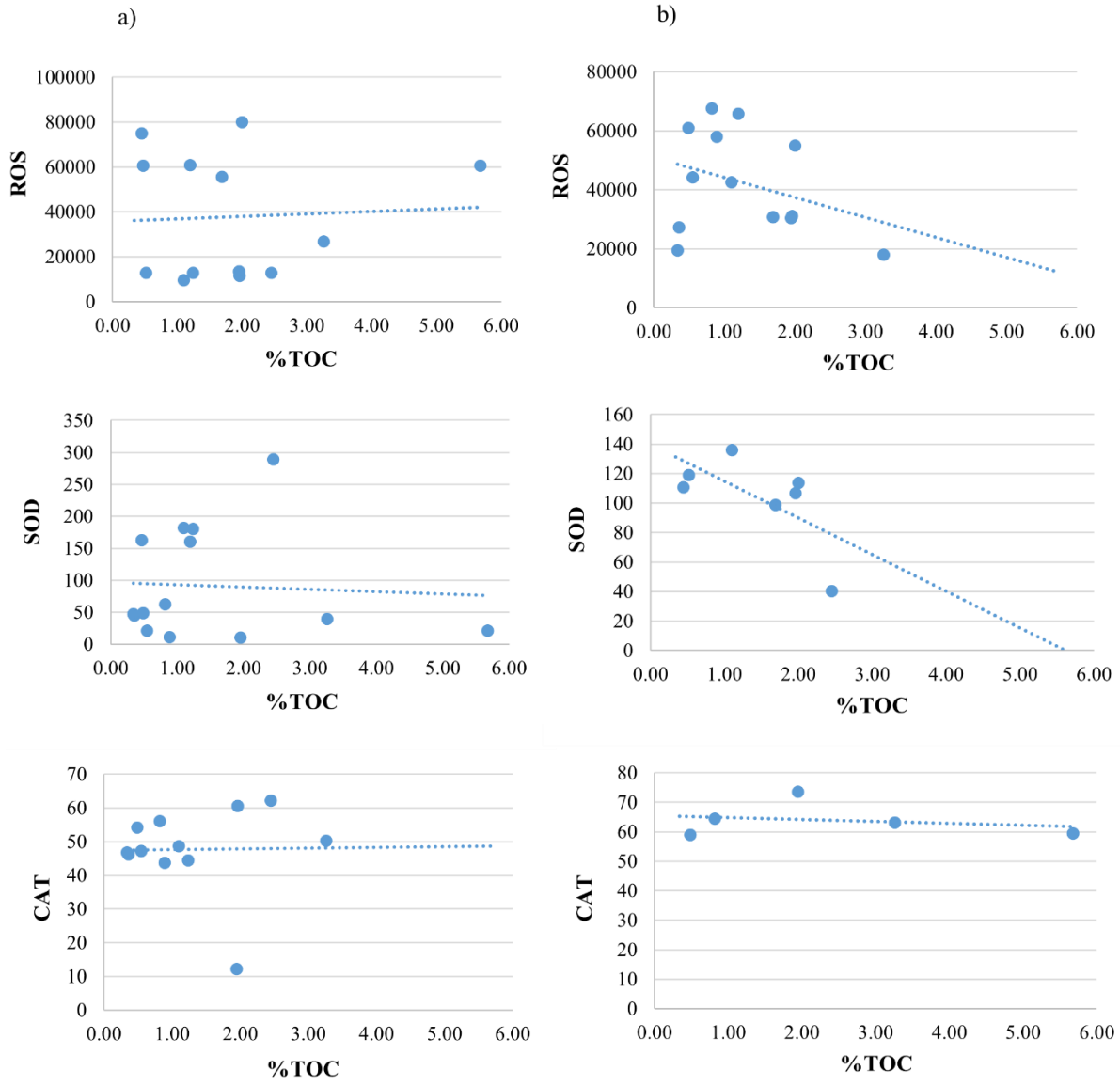

Figure 38 Correlation between the oxidative stress responses and %TOC in a) HuTu-80 and b) H4IIE-*luc* cells. CAT: catalase; SOD: superoxide dismutase; TOC: total organic carbon.

Table 17 Spearman's correlation analysis between the total organic carbon content and oxidative stress responses of the samples in the HuTu-80 and H4IIE-*luc* cell lines.

HuTu-80		**TOC**	**ROS**	**SOD**	**CAT**
Spearman's rho	Correlation coefficient (r_s)	1.000	-0.099	-0.029	0.273
	p-value	-	0.748	0.923	0.391
H4IIE-*luc*		**TOC**	**ROS**	**SOD**	**CAT**
Spearman's rho	Correlation coefficient (r_s)	1.000	-0.121	-0.536	0.100
	p-value	-	0.694	0.215	0.873

Correlation is statistically significant ($p \leq 0.05$; 2-tailed); CAT: Catalase: ROS: Reactive oxygen species; r_s: Spearman's correlation coefficient; SOD: Superoxide dismutase; TOC: Total organic carbon.

$\|r_s\| = 0$	No correlation
$\|r_s\| \leq 0.3$	Poor correlation
$0.3 < \|r_s\| \leq 0.5$	Fair correlation
$0.6 \leq \|r_s\| \leq 0.8$	Moderately strong correlation
$\|r_s\| > 0.8$	Very strong correlation

5.2 Cell viability as an endpoint of toxicity

Cytotoxicity is a non-specific endpoint of toxicity which can be used as an early warning sign since it captures and quantifies the toxic response elicited by all chemicals present in environmental mixtures (Escher *et al*., 2021). Cell death is reflected by a slowdown in metabolic activity (Belaid *et al*., 2021) and can be measured using the MTT cell viability assay which assesses mitochondrial function (Mosmann *et al*., 1983). In this study, the MTT cell viability bioassay was used to assess the cytotoxic potential of the highest three sample concentrations in four different cell lines: H4IIE-*luc*, MDA-kb2, T47D-KB*luc*, and HuTu-80. In some instances, concentration-dependent cytotoxicity was observed but overall, most of the samples were only cytotoxic at the highest concentration of 250 mg/mL. Since different cell lines originate from different organ tissues and these organs have different sensitivities towards environmental pollutants, the measured cytotoxic response is dependent on the cell type used (Belaid *et al*., 2021).

In this study, cytotoxicity was reflected differently in the four cells lines following exposure to the samples (i.e., soil extracts) (**Table 9–Table 14**). Consequently, the Kruskal-Wallis H and Mann-Whitney *U* tests were used to determine whether the differences in cell viability between the cell lines under investigation were statistically significant according to calculated Bonferroni adjusted *p*-value thresholds. Responses between the H4IIE-*luc* and T47D-KB*luc*; T47D-KB*luc* and MDA-kb2; and MDA-kb2 and HuTu-80 cell lines were found to be statistically significant (**Table 18**). Based on the cell viability results, this indicates that the rat liver H4IIE-*luc* (**Table 9**) and human breast MDA-kb2 (**Table 10–Table 11**) cells were the most sensitive to the soil extracts, followed by the human duodenum HuTu-80 cells (**Table 14**). The T47D-KB*luc* (human breast) cells had the highest resilience toward the samples (**Table 12–Table 13**). Chaufan

et al. (2014) demonstrated that the commercial glyphosate formulation, Roundup UltraMax, significantly decreased cell viability in the human liver HepG2 cells in a dose-dependent manner at concentrations far below agricultural recommendations (< 1 000 mg/mL). The response in the liver cells is not unexpected as the liver is the primary target for chemical-induced toxicity (Tabernilla *et al*., 2021) since it is located downstream of the GIT and receives the highest concentration of pollutants from the oral route (Escher *et al*., 2021). Apart from the responses of pesticides, previous studies have also evaluated cytotoxic responses elicited by environmental mixtures, including soil (Keita *et al*., 2021; Eze *et al*., 2022). The results of this study demonstrate the importance of including different types of cell lines for cytotoxicity assessment as it provides the true sum of the entire burden of environmental pollutants and covers different modes of action (Escher *et al*., 2021).

Table 18 Statistically significant differences between the cell viability of the four respective cell lines following exposure to the soil samples.

***p*-value rank**	**Pair**	**Bonferroni adjusted *p*-value threshold**	***p*-value (Mann-Whitney *U* test)**
15	H4IIE-*luc* (AhR agonism) & T47D-KB*luc* (ER antagonism)	0.003	0.001*
14	T47D-KB*luc* (ER agonism) & MDA-kb2 (AR agonism)	0.003	0.001*
13	T47D-KB*luc* (ER agonism) & MDA-kb2 (AR antagonism)	0.003	0.001*
12	T47D-KB*luc* (ER antagonism) & MDA-kb2 (AR agonism)	0.003	0.001*
11	T47D-KB*luc* (ER antagonism) & MDA-kb2 (AR antagonism)	0.003	0.001*
10	H4IIE-*luc* (AhR agonism) & T47D-KB*luc* (ER agonism)	0.005	0.003*
9	MDA-kb2 (AR agonism) & HuTu-80	0.006	0.006*
8	MDA-kb2 (AR antagonism) & HuTu-80	0.006	0.009
7	T47D-KB*luc* (ER agonism) & HuTu-80	0.007	0.050
6	T47D-KB*luc* (ER antagonism) & HuTu-80	0.008	0.073
5	H4IIE-*luc* (AhR agonism) & MDA-kb2 (AR agonism)	0.010	0.105
4	H4IIE-*luc* (AhR agonism) & HuTu-80	0.013	0.146
3	H4IIE-*luc* (AhR agonism) & MDA-kb2 (AR antagonism)	0.017	0.330

p-value rank	Pair	Bonferroni adjusted *p*-value threshold	*p*-value (Mann-Whitney *U* test)
2	MDA-kb2 (AR agonism) & MDA-kb2 (AR antagonism)	0.025	0.424
1	T47D-KB*luc* (ER agonism) & T47D-KB*luc* (ER antagonism)	0.050	1.000

*Statistically significant compared to Bonferroni adjusted p-value threshold.

5.3 Instrumental chemical analysis

As in the case of the soil extractions for the *in vitro* bioassays, polar compounds were extracted from the soil using pH-adjusted deionised water and chemically screened for the presence of pesticides. However, no pesticides were detected most likely since these samples had not been concentrated and is not further discussed. Although *in vitro* bioassays can quantify effects of environmentally low concentrations, this is not always the case for all analytical instruments (Horn *et al*., 2020). Therefore, methanol was used as the extraction solvent to specifically target polar compounds and because it shows better pesticide recovery compared to water (Atalay & Hwang, 1999; Guzzella & Pozzoni, 1999). As mentioned in Chapter 2 (Sections 2.2.1–2.2.4), current-use pesticides have been detected in the South African environment, including 2,4-D, atrazine, dicamba and imidacloprid. Numerous studies have also reported on the endocrine disruptive effects, oxidative stress, and AChE inhibition associated with these pesticides in non-target organisms. Based on this premise and the biological effects observed (Section 4.2), instrumental chemical analysis was used to detect the concentration of these four pesticides (quantitative) and screen for the presence other agrochemicals (qualitative) in the soil samples.

5.3.1 Quantitative and qualitative analysis of agrochemicals in samples

All four the pesticides atrazine, 2,4-D, dicamba, and imidacloprid were quantifiable in at least one of the samples (**Table 16**). Quantifiable amounts of atrazine were detected in most of the soil samples, with atrazine having the highest detection frequency of 89% (**Table 16**). This was expected as most of the sampling locations were maize fields and in South Africa atrazine is extensively applied to maize crops (Dabrowski, 2015b). Overall, the levels of atrazine in this study (0.2–208.6 ng/g) were similar to those reported by Sun *et al*. (2017) (< 1.0–113 ng/g) in agricultural soils from rice field paddies, mulberry, vegetable, and maize fields in the area of the Yangtze River Delta in China. However, the two maximum atrazine concentrations (161.6 and 208.6 ng/g; M14 and M8, respectively; **Table 16**) were considerably higher compared to other literature. Riedo *et al*. (2021) detected atrazine in the range of 0.1–50 ng/g in agricultural soil (arable and vegetable farming) sampled from Switzerland. During the assessment of triazine herbicides in arable soil from China, the maximum atrazine concentration was 73.80 ng/g (Wang & Liu, 2020). However, there were some exceptions. Degrendele *et al*. (2022) collected soil samples from agricultural areas—Grabouw and the Hex River Valley—in the Western Cape province of South Africa, which were analysed for the presence of 30 current-use pesticides, including atrazine. The detected

concentrations of atrazine in soil were considerably lower compared to those found in the present study: 8.6–22.6 pg/g for the Hex River Valley and 15.5 pg/g for Grabouw. These lower concentrations of atrazine are likely attributed to the type of crops cultivated in the sampling areas. Atrazine is mainly applied to maize, while the Hex River Valley and Grabouw are dominated by the cultivation of table grapes and pome fruits, respectively (Curchod *et al*., 2020). Furthermore, the lowest atrazine concentration reported in this study (0.2 ng/g) was the same as the level of atrazine detected in topsoil sampled in 2014 from agricultural fields in western Germany where atrazine has been banned since 1991. This indicates that atrazine seems to have a longer half-life in soil than initially anticipated and the long-term behaviour of atrazine in soil needs to be reconsidered (Vonberg *et al*., 2014).

Although there are no soil quality guidelines for pesticides in South Africa, the levels of atrazine quantified in the present study exceeded the environmental quality standard of Latvia for atrazine in soil (0.2 ng/g) but was lower than the Dutch soil intervention value for atrazine (710 ng/g) (Innovative Sustainable Remediation, 2017). Furthermore, the presence of atrazine in the water-soluble fraction of all the agricultural soil samples of the present study, highlights the potential of this pesticide leaching into non-target aquatic environment. In South Africa, atrazine is one of only two pesticides—endosulfan being the other—for which there are water quality guidelines. The water quality guideline for atrazine in aquatic ecosystems is 10 µg/L as stipulated by the Department of Water Affairs and Forestry (DWAF, 1996). However, this guideline has not been updated since 1996, and there are also no guidelines for agricultural water used for irrigation or livestock watering.

In the current study, 2,4-dichlorophenoxyacetic acid was detected in the majority of the samples (74%) (**Table 16**). Approximately 60% of 2,4-D used in South Africa is applied to maize crops (Dabrowski *et al*., 2014) and this could account for the high detection frequency. The levels of 2,4-D in this study (4.5–832.4 ng/g) could not be compared to other literature due to the lack of studies reporting on the presence of 2,4-D in soil. 2,4-Dichlorophenoxyacetic is easily soluble in water, making it a highly effective herbicide that rapidly penetrates through plant leaves or roots (Peterson *et al*., 2016). It is therefore directly applied to soil or crops. However, this can be problematic for the environment as it is prone to run-off or leach through soil due to its polarity, with approximately 91.7% of 2,4-D eventually ending up in waterbodies (Mountassif *et al*., 2008). Although 2,4-D has been reported to be present in South African aquatic environment (Horn *et al*., 2019), no studies have quantified it in soil. This is in all probability due to the herbicide's non-persistent nature. In soil, 2,4-D has a half-life of only 5 days and its amine salts dissociate in less than 3 minutes under most environmental conditions (Wilson *et al*., 1997). Moreover, 2,4-D is known to undergo microbial degradation in soil (Islam *et al*., 2018). This could explain the absence of quantifiable amounts (< LOQ; **Table 16**) of it in some of the soil samples as several bacteria and fungi species isolated from soil have been found to degrade 2,4-D by using it as a carbon and energy source (Cycoń *et al*., 2011; Itoh *et al*., 2013; Xia *et al*., 2017).

Although dicamba had a high detection frequency (84%) (**Table 16**), the lowest recovery was reported for it (22.08%) (**Table 15**), and therefore a considerable amount of dicamba was perhaps not extracted. While the highest pesticide concentration quantified in this study was for dicamba (8 105.0 ng/g), no comparable studies were found in literature. Likely this may be due to dicamba's high volatility in the environment following application. Dicamba volatilisation represents an important exposure route towards non-target areas (Bish *et al*., 2019; Oseland *et al*., 2020) and might explain its absence in areas of known application. Even though dicamba is known to be applied to maize crops in South Africa, the herbicide was not even mentioned in a publication by Dabrowski *et al*. (2014) about the prioritisation of agricultural pesticides used in South Africa. This is worrisome as the level of dicamba detected in this study shows contamination of soil. Based on this, the presence of dicamba in the South African environment warrants further investigation.

The use of imidacloprid as a seed coating could explain the low detection rate of the insecticide in the samples (32%; **Table 16**). Moreover, in South Africa, only 11.5% of imidacloprid is used in maize cultivation (Dabrowski *et al*., 2014). Therefore, the low usage of imidacloprid in South African maize growing regions could be another factor contributing to the low detection frequency. Nonetheless, the levels of imidacloprid in soil reported in this study (19.5–96.9 ng/g) are mostly comparable with previous literature. Humann-Guilleminot *et al*. (2019) reported a maximum imidacloprid concentration of 29.7 ng/g in soil from conventional farming practices in Switzerland. Dankyi *et al*. (2014) collected soil from several cocoa farms in Ghana and investigated the presence of five neonicotinoids, including imidacloprid, which was found to be the predominant insecticide present. Imidacloprid concentration ranged from 4.3–251.4 ng/g (Dankyi *et al*., 2014). However, there are some exceptions where the concentrations detected in the present study are higher than previous findings. Pook and Gritcan (2019) found imidacloprid concentrations between 0.5 and 9.4 ng/g in maize fields from New Zealand. Moreover, all the imidacloprid concentrations quantified in the present exceeded the New Zealand Environmental Protection Agency's Environmental Exposure limit of 1 ng/g (**Table 16**). Schaafsma *et al*. (2015) also investigated the concentration of neonicotinoids associated with maize farming. Although the mean total neonicotinoid residues after planting were 0.53–38.98 ng/g, imidacloprid was only detected at one sampling location with a concentration equal to the LOD (0.01 ng/g) (Schaafsma *et al*., 2015).

The data obtained following UHPLC-QTOF/MS analysis was also used to screen for the presence of other agrochemicals. Based on the results obtained, several other compounds were also detected, especially herbicides and insecticides (**Figure 36** and **Figure 37**). Da Silva *et al*. (2021) reported the presence of certain pesticides in groundwater and soil from sugarcane crops in Brazil. Suspect screening of more than 7 800 compounds allowed the tentative identification of 57 compounds in the Brazilian study, some of which were also detected in the soil samples of this study: metamitron, pirimicarb, aminocarb, and propiconazole (**Tables S13–S14**). Although the biological effects observed during the present study cannot be directly attributed to the plethora of agrochemicals present in the samples, qualitative screening is useful

tool to identify contaminants of interest. However, to confirm the presence of specific agrochemicals, the extraction and quantification methods must be optimised for these specific compounds of interest.

Table 19 Summary of the statistically and/or practically significant results reported on in Chapter 4.

Biological effect				Xenobiotic metabolism	Endocrine disruption				Oxidative stress and damage								Damage to the NNCS	
Cell line				H4IIE-*luc*	MDA-kb2		T47D-KB*luc*		HuTu-80				H4IIE-*luc*				HuTu-80	H4IIE-*luc*
Sample name	Irrigation practice	TOC	Soil type	AhR agonism	AR agonism	AR antagonism	ER agonism	ER antagonism	ROS	SOD content	CAT activity	LPO	ROS	SOD content	CAT activity	LPO	AChE activity	
Mpumalanga province sampling locations																		
M1	R	ML	S	-	-	✓	-	-	↑	↑	-	-	↑	-	-	-	-	-
M2	R	L	S	-	-	-	-	-	↑	-	-	-	-	↑	-	-	-	-
M3	R	L	S	-	-	-	-	-	↑	↑	-	-	-	-	-	-	-	-
M4	R	ML	S	-	-	✓	-	-	↑	-	-	-	↓	↑	-	-	-	-
M5	R	ML	S	-	-	-	-	-	↑	-	-	-	↑	↑	-	-	-	-
M6	R	L	S	-	-	-	-	-	↓	-	-	-	-	↑	-	-	-	-
M7	R	ML	S	-	-	✓	-	-	↓	↑	↑	-	↑	↑	-	-	-	↑
M8	R	ML	S	-	-	✓	-	-	↓	↓	↑	-	↓	↑	-	-	-	-
M9	R	ML	G	-	-	✓	-	-	↓	-	↓	-	↓	-	↑	-	-	-
M10	R	ML	G	-	-	✓	-	-	↓	↑	↑	-	-	-	-	-	-	↓
M11	R	M	S	-	-	-	-	-	↓	↑	↑	-	-	↓	-	-	-	-
Vaalharts Valley, Northern Cape province sampling locations																		
M12	R	L	S	-	-	-	-	-	-	↓	↑	-	↑	-	↑	-	-	↓
M13	P	L	S	-	-	✓	✓	✓	-	↓	↑	-	↑	-	-	-	-	↓
M14	R	L	S	-	-	-	-	-	-	↓	↑	-	↑	-	↑	-	-	↓
M15	R	L	S	-	-	-	-	-	-	↓	↑	-	↓	-	-	-	-	-
P1	N	H	S	-	-	-	-	-	↑	↓	-	-	-	-	↑	-	-	-
P2	N	L	S	-	-	-	✓	-	-	↓	↑	-	↑	-	-	-	-	-
P3	N	M	S	-	-	-	✓	-	↓	↓	↑	-	↓	-	↑	-	-	-
P4	N	L	S	-	-	✓	-	-	-	↓	↑	-	↓	-	-	-	-	-

AChE: Acetylcholinesterase; AhR: Aryl hydrocarbon receptor; AR: Androgen receptor; CAT: Catalase; ER: Oestrogen receptor; LPO: Lipid peroxidation; NNCS: Non-neuronal cholinergic system; ROS: Reactive oxygen species; SOD: Superoxide dismutase; ✓ Present; ↑ Increase; ↓ Decrease.

Irrigation practice

R	Rainfed cultivation
P	Pivot irrigation
N	Non-pivot irrigation

Total organic carbon content

L	Low
ML	Moderately low
M	Medium
H	High

Soil classification

C	Clay
S	Sand
G	Gravel

Biological effects

-	No significant response
✓	Endocrine disruptive effects
↑↓	Evidence of oxidative stress and/or damage
↓	Evidence of antioxidative effects
↑↓	Effects on the non-neuronal cholinergic system

5.4 The presence of polar AhR ligands

Aryl hydrocarbon receptor ligands are usually non-polar, dioxin-like compounds, which include halogenated-dioxins (e.g., TCDD), halogenated aromatic hydrocarbons (HAHs), PAHs, and PCBs (Nguyen & Bradfield, 2008; Stejskalova *et al*., 2011; Tian *et al*., 2015; Doan *et al*., 2019; Cha *et al*., 2022). Due to their hydrophobicity and lipophilicity, these dioxin-like compounds adsorb tightly to soil particles and lipids. In order to extract dioxin-like chemicals from environmental matrices, non-polar extraction solvents such as dichloromethane and hexane are required. Based on this premise, it is thought that water-soluble (i.e., polar) compounds do not activate the AhR. However, it has recently been shown that polar compounds can induce the AhR genomic signalling pathways (Cha *et al*., 2022). Therefore, the H4IIE-*luc* bioassay was used to investigate whether extracts from maize and pecan soils in South Africa contain polar AhR agonists. The focus of this study was the water-soluble fraction of agricultural soils since it represents the fraction of soil which would reach non-target areas under realistic environmental conditions. In other words, the compounds which would run-off or leach from the soil following rainfall or irrigation.

Agricultural soils are direct recipients of agrochemicals and may show bioactivity towards the AhR (Celma *et al*., 2021). However, at the concentrations investigated in this study, none of the samples caused AhR activation (**Table 9** and **Table 19**). This is likely due to the absence of classical AhR agonists in the samples since the extraction method followed in the present study excluded non-polar compounds. Nevertheless, it has recently been evidenced that polar compounds can bind to the AhR. Cha *et al*. (2022) used the H4IIE-*luc* bioassay to investigate AhR agonism in extracts prepared from the liver of black-tailed gulls. The polar fraction caused significant AhR-mediated effects and by using effects-directed analysis several novel polar AhR agonists were identified including corticosterone, a well-known glucocorticoid, and oxadixyl, a systematic fungicide used as a seed treatment to prevent phytophthora pests causing root rot in tomatoes and maize. The AhR potencies of the polar fraction were also greater than that of the non-polar fraction (Cha *et al*., 2022). However, even though oxadixyl was detected in some of the soil samples (M4, M6, M7, and M10) in the present study (**Tables S13–S14**), no AhR agonistic responses were elicited (**Table 9** and **Table 19**). Aryl hydrocarbon receptor ligand activity was also found in the polar fractions of road dust and diesel exhaust particulates. Based on these results, it is suggested that many unknown AhR ligands are present in the polar fraction of different matrices (Misaki *et al*., 2008). In another study, organic extracts (lipophilic molecules) of humic substances naturally found in the organic fraction of soil elicited AhR agonistic activity in comparison to the reference compound, TCDD. After extraction of the hydrophobic and lipophilic compounds with hexane and dichloromethane, the authors also investigated the AhR activity of the remaining extraction residues. Interestingly, higher luciferase activity was observed for these residues, suggesting the presence of polar AhR agonists (Janošek *et al*., 2007).

The results of this study do not necessarily indicate the complete absence of polar AhR agonists in the soil samples. It is possible that non-classical AhR ligands (e.g., polar compounds) are weak activators of the

AhR due to their low binding affinity compared to classical dioxin-like compounds (e.g., TCDD and HAHs). Long-term continuous exposure to low-affinity ligands can cause AhR agonistic activity (Janošek *et al*., 2007) and not necessarily only 72 hours of exposure as in the case of this study. Moreover, this study only assessed AhR agonism and not antagonism since it is mostly unknown whether AhR antagonistic activity would cause adverse health effects by interfering with the normal functioning of the AhR. However, some studies have found that certain pesticides cause AhR antagonism. When investigating the agonistic and antagonistic AhR activity of 29 persistent organic pollutants (POPs)—including the pesticides dieldrin, chlordane, and hexachlorobenzene—most of the POPs caused AhR antagonism both as single compounds and in a mixture (Doan *et al*., 2019). The absence of AhR agonistic activity in 189 out of the 200 pesticides investigated by Takeuchi *et al*. (2008) and covering several classes—acid amides, carbamates, triazines, organochlorine pesticides (OCPs), organophosphates (OPs), and pyrethroids—highlights the potential for antagonism which was not investigated in the present study. This is especially the case since approximately 33% of the 189 pesticides which did not show AhR agonism were present in die soil samples of this study (**Tables S13–S14**). Based on this, AhR antagonism of the water-soluble fraction of agricultural soil could be a potential avenue for future research.

5.5 Endocrine disruptive effects

Endogenous steroid hormones regulate key processes related to sexual development. As a result, many xenobiotics are EDCs that mimic endogenous hormones, causing adverse effects in exposed non-target biota. This could contribute to the onset of reproductive disorders (Wilson *et al*., 2002; Ermler *et al*., 2010; De Falco *et al*., 2015). Although studies have investigated the endocrine-disrupting effects in different environmental matrices such as water (Aneck-Hahn *et al*., 2008; Leusch *et al*., 2018), sediment (Urbatzka *et al*., 2007; Alvarez-Muñoz *et al*., 2015) and tissue (Arukwe *et al*., 2016), little attention has been paid to the endocrine disrupting effects of soil (Li *et al*., 2015; Zhang *et al*., 2018). This is worrisome as soil can act as a "sink" for EDCs since these pollutants attach to the soil particles due to the presence of clay minerals and humic materials (Li *et al*., 2015). Since many pesticides are known EDCs (Baines *et al*., 2017; Martyniuk *et al*., 2020), it is important to assess the endocrine disruptive effects associated with agricultural soils due to the large input of agrochemicals. In this study, various endocrine disruptive endpoints were assessed *in vitro* following exposure to the water-soluble fraction of maize and pecan soils.

5.5.1 (Anti-)androgenicity

The MDA-kb2 cell line endogenously expresses the AR and GR which are ligand-dependent transcription factors (Wilson *et al*., 2002). The genomic pathway of the AR and GR are activated by the binding of endogenous androgens and glucocorticoids, respectively (Kulik *et al*., 2021). In this study, the MDA-kb2 bioassay was used to assess the AR and/or GR activity of the polar compounds present in the maize and pecan soils. These cells were selected since they show sufficient hormonal sensitivity for receptor agonists and antagonists (Wilson *et al*., 2002) and have been successfully applied for the screening of environmental

chemicals for especially (anti-)androgenic effects (Blake *et al*., 2010; Aït-Aïssa *et al*., 2010; Ma *et al*., 2019).

Although individual androgen agonists can cause additive effects when in mixtures (Blake *et al*., 2010) this was not the case in this study. During the MDA-kb2 bioassay, none of the samples (MP or VH) showed any concentration-response relationship during the assessment of AR agonism (**Table 10** and **Table 19**). These results indicate that the samples most likely do not contain any polar compounds at a concentration to cause agonism of the AR and/or GR. The absence of *in vitro* androgenic activity in this study is in line with the findings reported in literature for various pesticides (Andersen *et al*., 2002; Kojima *et al*., 2004; Xu *et al*., 2008; Aït-Aïssa *et al*., 2010). Kojima *et al* (2004) evaluated AR-mediated effects of 200 pesticide active ingredients covering several classes, including carbamates, OCPs, OPs, pyrethroids, and triazines using Chinese hamster ovary cells (CHO-K1). Although 2,4-D, atrazine, and imidacloprid were also evaluated, these compounds did not cause AR agonism. The lack of androgenic activity was also reported by Aït-Aïssa *et al*. (2010) for atrazine, benomyl, diuron, fenarimol, methyl parathion, and propiconazole when using the MDA-kb2 cell line. All seven of these pesticides are registered for use in the South African agriculture sector (Quinn *et al*., 2011; Dabrowski, 2015c) and were also detected in some of the maize and pecan soil samples from MP and VH (**Tables S13–S14**). Based on this, the mechanism of action of the polar compounds present in the maize and pecan soil could be AR antagonism rather than agonism. This is expected as environmental compounds are more likely to interfere with the AR through anti-androgenicity than androgenicity (Conley *et al*., 2017). This would also explain why no androgenic activity was observed in this study.

Alternatively, the absence of androgen agonism in this study (**Table 10** and **Table 19**) could be explained by masking effects. According to Kim *et al*. (2006), most environmental anti-androgens antagonise the action of androgens via competition with the AR or by reducing the transcriptional activation of target genes (Kim *et al*., 2006; Wilson *et al*., 2008). Several authors have reported that anti-androgens mask the effects of androgens during *in vitro* bioassays (Weiss *et al*., 2009; Urbatzka *et al*., 2007; Alvarez-Muñoz *et al*., 2015). According to Alvarez-Muñoz *et al*. (2015) the activity of AR agonists present at low concentrations were masked by AR antagonists present in higher concentrations within sediment extracts. However, this masking effect is only likely to occur when the anti-androgens are present in very high concentrations (Blake *et al*., 2010) or when the anti-androgens are more potent than the androgens. Environmental matrices, especially soil, contain low concentrations of a wide variety of chemical classes with different physicochemical properties (Li *et al*., 2015). Nevertheless, the possibility exists that potent AR antagonists masked the effect of weaker androgens, especially since several samples showed anti-androgenicity (**Table 11**; **Table 19**; **Figure 22**).

Another potential explanation for the absence of AR agonism is the interference of medium constituents with the receptor's response to other steroidal hormones. Oestrogens, progestogens and mineralocorticoids can all bind to the AR (Soto *et al*., 2006). Especially, E_2 is known to bind to the AR with low affinity, interfering with the MDA-kb2 cell's response to agonists (Christiaens *et al*., 2005; Blake *et al*., 2010). Phenol red mimics the activity of oestrogens (Berthois *et al*., 1986; Węsierska-Gądek *et al*., 2007), and many oestrogenic compounds are also known anti-androgens (Sohoni & Sumpter, 1998). Consequently, it is possible that the phenol red—present in the nutrient medium (L-15 medium) used to cultivate the MDA-kb2 cells during this study—evoked oestrogenic and/or anti-androgenic responses. This could have interfered with the AR's response to samples (Ermler *et al*., 2010). However, this interference did not influence the results of the PC (testosterone) (**Figure 20**). Nonetheless, the interference could have been enhanced in the wells that received the environmental extracts containing numerous unknown compounds that could have caused a synergistic anti-androgenic effect.

For the past 20 years, much attention had been paid to chemicals that act as agonists—of especially the ER and AR—with less attention to those acting as antagonists (Leusch *et al*., 2017). This is worrisome as anti-androgens are the most common type of EDCs (Weiss *et al*., 2009; Orton *et al*., 2018). In recent years, there has been increasing interest in assessing the antagonistic activity of environmental samples (Leusch *et al*., 2017). Yet, there is still little information about AR antagonists in soil (Li *et al*., 2015).

As a rule of thumb, antagonism is assessed in the presence of a constant concentration of a known agonist, with antagonists present in samples supressing the known agonist's signal (Escher *et al*., 2021). Consequently, the results of an anti-androgenic bioassay are dependent on the concentration of the AR agonist used. The response of weak AR antagonists in an environmental sample could be overlooked if the agonist concentration used is too high, while very low agonist concentrations could affect the dynamic range of the assay (Ermler *et al*., 2010). For the assessment of antagonism *in vitro*, Neale and Leusch (2015) recommend an agonist background concentration which causes 80% of the maximum effect (i.e., 80% activation of the receptor in question). This will optimise the sensitivity and reproducibility of the bioassay (Neale & Leusch, 2015). Concentrations responding to the EC_{80} of a known agonist has also been used by other authors when investigating antagonistic effects (Celma *et al*., 2021). The agonist concentration used in this study (0.283 ng/mL of testosterone) also corresponds to the EC_{80} for AR agonism and is considered suitable since it allowed for the detection of anti-androgenicity of the samples.

For the assessment of AR antagonism, flutamide was used as the reference compound. The IC_{50} (0.66 µg/mL) obtained from the flutamide concentration-response curve in this study is comparable to the IC_{50} (0.43 ug/mL) obtained for flutamide by Ermler *et al*. (2010) who investigated the sensitivity of an adjusted MDA-kb2 bioassay as a screening tool for anti-androgens with high reproducibly. The slight difference could be attributed to the potency of the agonist used. In this study testosterone was used, while Ermler *et*

al. (2010) used DHT which is a considerably stronger AR agonist (Blake *et al*., 2010). A stronger agonist could reduce the inhibitory effect of the antagonist, increasing the IC_{50} of the AR antagonist.

In contrast to the assessment of receptor agonism, the results of receptor antagonism are more susceptible to interference from cytotoxicity (Neale & Leusch, 2015). It is therefore especially important to run a cytotoxicity assay in parallel to an inhibition bioassay to monitor baseline toxicity (Escher *et al*., 2021). This will ensure that if a reduction in luciferase activity is observed it can be attributed to true antagonism and not masked cytotoxicity (Escher *et al*., 2021). In the present study, results obtained for the MTT cell viability assay indicated that sample concentrations used for determining anti-androgenicity were mostly not cytotoxic towards the MDA-kb2 cells (**Table 11** and **Table 19**). Accordingly, any reduction in luciferase activity was therefore attributed to the presence of AR antagonists in the samples and not cytotoxic responses.

Conversely to the absence of androgenic activity, 42% of the samples in this study exhibited statistically significant anti-androgenic activity (FC < 1), and six of the eight (M1, M4, M7, M8, M9, and M10) (**Table 19**) are from MP. The results of this study were comparable with the findings of Li *et al*. (2015) where 54% of soil samples from an agricultural area (surrounding the Second Songhua River in China) induced anti-androgenic activity. Since the majority of the MP samples induced AR antagonism, it could likely be due to the presence of certain pesticides in these samples. Of the four pesticides quantified (**Table 16**), atrazine was the only compound detected in all the MP samples that showed anti-androgenic effects (**Figure 22**). 2,4-Dichlorophenoxycetic acid, dicamba and imidacloprid were only quantified in some of the MP samples showing AR antagonism (**Table 16**).Of these three pesticides, dicamba was previously found to cause AR antagonism in the yeast anti-androgen screen assay (Westlund & Yargeau, 2017), while exposure to imidacloprid significantly decreased expression of the AR in male mice (Yuan *et al*., 2020). However, even though 2,4-D significantly enhanced testosterone activity via the AR, the herbicide did not show any AR antagonistic activity in African green monkey kidney (Vero) cells following 24 hours of exposure (Sun *et al*., 2012). The highest atrazine concentration (208.6 ng/g; **Table 16**) was reported for M8 which also showed the most AR antagonistic activity (lowest FC value) in the present study (**Figure 22**). Several endocrine disruptive effects have been reported for atrazine, including the inhibition of testosterone production in the Leydig cells of male rats (Friedmann, 2002); reproductive dysfunction in adult zebrafish (*Danio rerio*) (Wirbisky *et al*., 2016); and feminisation in male African clawed frogs (*Xenopus laevis*) (Hayes *et al*., 2010).

On the other hand, of the 42% of samples that elicited AR antagonism during the present study only two of the samples (M13 and P4) were from the VH (**Table 11**; **Table 19**; **Figure 22**). However, an interesting observation is that although P4 had the lowest atrazine concentration, it showed the second most potent anti-androgenicity after M8 (**Table 16** and **Figure 22**). This could indicate that other pesticides, and not

necessarily atrazine, might be responsible for the observed anti-androgenicity. Together with atrazine, dicamba was the only other pesticide also quantified in both the VH samples (M13 and P4) that exhibited AR antagonism (**Table 16** and **Figure 22**). A study by Westlund and Yargeau (2017) investigated the endocrine disruptive effects of various herbicides, insecticides, and fungicides found in the effluent of wastewater treatment plants (WWTPs). Results showed that dicamba showed a slightly positive response for anti-androgenic activity during the yeast anti-androgen screen assay. Even though dicamba is known to be applied to maize crops in South Africa, the herbicide was not even mentioned in a publication by Dabrowski *et al*. (2014) about the prioritisation of agricultural pesticides used in South Africa. The maximum dicamba concentration (8 105.0 ng/g) was the highest quantified for all four the pesticides (**Table 16Table 16**). This is worrisome as the level of dicamba detected in this study shows contamination of soil. Based on this, the presence of dicamba and associated biological effects in the South African environment warrants further investigation.

Androgen receptor antagonism has also been reported for several other pesticides and pesticide mixtures. Ma *et al*. (2019) investigated the individual and combined anti-androgenic activity of twelve agricultural pesticide active ingredients individually and in combination also using the MDA-kb2 cell line and found that all the pesticides exhibited anti-androgenicity. Fenitrothion exhibited the most potent anti-androgenicity, followed by dimethomorph, difenoconazole, bromoporpylate, prochloraz, imazalil, and endosulfan; while cyhalothrin, dicofol, dimethomorph, procymidone, and vinclozolin showed lower anti-androgenic activity. Eight (cyhalothrin, dicofol, fenitrothion, endosulfan, imazalil, prochloraz, procymidone, and vinclozolin) and three (endosulfan, prochloraz, and vinclozolin), of the twelve pesticides were included in the studies by Kojima *et al*. (2004) and Aït-Aïssa *et al*. (2010), respectively. However, only two of these pesticides, cyhalothrin and procymidone which previously showed low AR antagonism (Ma *et al*., 2019), were detected in the soil samples of the present study (**Tables S13–S14**). Although, previous research provides evidence of anti-androgenicity of environmental pollutants, Leusch *et al*. (2017) stated that the comparison of antagonist data obtained in one study with other literature can be challenging due to the interference of experimental artefacts. An example of this is the presence of dissolved organic carbon in samples which can reduce the bioavailability of the agonist and influence the results obtained for antagonism (Neale *et al*., 2015).

Environmental mixtures contain many individual compounds with bioactivities ranging from non-toxic to very potent. Based on this it is possible that not all chemicals are granted access to bind to the AR. This was evidenced by Archer and Van Wyk (2015) when assessing the AR activity of several pesticide active ingredients commonly used in the Western Cape province of South Africa using the recombinant yeast anti-androgen screen. Individually, chlorpyrifos, dimethomorph, fenarimol, folpet, mancozeb, procymidone, and vinclozolin caused anti-androgenic responses in a dose-dependent manner. However, when these active ingredients were combined to test whether a mixture would cause an additive response, lower IC_{50} values

were obtained compared to those of the individual active ingredients (Archer & Van Wyk, 2015). Yet, this does not seem to support the results of the present study. Since the responses for AR antagonism cannot be attributed to individual pesticides with absolute certainty, the samples in this study most likely contained a mixture of AR antagonists which acted jointly on the AR when combined at low concentrations causing the observed anti-androgenic effects (Ma *et al*., 2019). This might have been the case for the polar compounds present in M10, since AR antagonism was reported for M10 (**Figure 22**), although this sample had the second lowest pesticide load (ΣPesticides = 32.8 ng/g; **Table 16**) of all the soil samples. It is also possible for environmental samples to contain a mixture of compounds with either androgenic or anti-androgenic activity; or compounds which exhibit both androgenic and anti-androgenic activity, for example linuron (Christiaens *et al*., 2005). Linuron was detected in three of the maize soil samples (**Table S13**), with two of the samples (M7 and M8) showing anti-androgenicity (**Figure 22**). This is likely explained by the presence of partial agonists in these samples (Weiss *et al*., 2009). Partial agonists can bind to and activate the AR. However, the responses of partial agonists are usually lower compared to the responses of true AR agonists. Therefore, partial agonists can act as anti-androgens by inhibiting the effect of true agonists (Weiss *et al*., 2009).

5.5.2 Glucocorticoid receptor agonism

Apart from androgenic activity, the MDA-kb2 cell line also enables the quantification of the potency of GR agonists or antagonists based on the intensity of luminescence (Blake *et al*., 2010). Glucocorticoids are a class of steroid hormones named after their role in glucose metabolism (Kulik *et al*., 2021) and have many important physiological functions (Chang *et al*., 2007). Since the MDA-kb2 cell line expresses both the AR and GR (Wilson *et al*., 2002), two bioassays are usually performed for the assessment of agonism. During the first assay, agonism is assessed without blocking any receptors. When agonistic activity is observed it could indicate either AR or GR agonism. To distinguish between the presence of AR or GR ligands, a second bioassay is performed during which the AR is blocked with a known antagonist such as flutamide, which only blocks the AR (Wilson *et al*., 2002). This leaves the GR open for ligand binding. Therefore, when agonism is detected during this second bioassay, the response measured can be attributed to the presence of GR agonists. If no response is elicited when blocking the AR, the initial increase in luciferase activity is attributed to AR agonism.

Although androgens and oestrogens are the most common classes of steroidal hormones that affect environmental health, glucocorticoids have become a major concern in recent years due to their widespread detection in the environment (Weizel *et al*., 2018; Shen *et al*., 2020). Based on the results obtained for the MDA-kb2 activation bioassay, no agonism was detected for any of the samples, indicating no AR and/or GR ligands (**Table 10** and **Table 19**). The absence of GR agonists could likely be attributed to the nature and use of glucocorticoids. Natural glucocorticoids are released in mammals in a complex circadian and ultradian manner (Stavreva *et al*., 2012) and their activity is mediated through the GR and/or

mineralocorticoid receptor (Bigas *et al*., 2018). However, synthetic glucocorticoids are widely used as pharmaceuticals to treat asthma, severe allergies, arthritis, and skin conditions due to their anti-inflammatory and immunosuppressive properties (Chang *et al*., 2007; Stavreva *et al*., 2012; Khammissa *et al*., 2016). Consequently, mixtures of steroid hormones, including glucocorticoids, originating from hospital waste and household sewage enter WWTPs (Weizel *et al*., 2018; Yazdan *et al*., 2021). Wastewater treatment plants are not designed to remove glucocorticoids (Stavreva *et al*., 2012) and to date no management technologies have been developed yet (Yazdan *et al*., 2021). Consequently, these synthetic chemicals are discharged into water sources, causing widespread environmental contamination (Stavreva *et al*., 2012). As WWTPs are considered the main source of chemicals that act as glucocorticoids, it might explain the absence of glucocorticoid activity since the samples for this study were collected from agricultural areas. Previous literature has reported that GR disruptive effects in agricultural soil were not related to the presence of pesticides (Zhang *et al*., 2018). However, there are no WWTPs in the close vicinity of the sampling locations from where glucocorticoids could originate. Nonetheless, since the water used to irrigate crops in the VH originate from the Vaal River (Van Rensburg *et al*., 2011; Verwey & Vermeulen, 2011), any pollution of the river could influence the quality of water used to irrigate crops. In South Africa it is well-known that the Vaal River is heavily polluted. As recent as 2018 raw sewage was found to be discharging into the Vaal River due to burst sewer pipes and dysfunctional WWTPs (Mnguni, 2022). Therefore, any effects originating from WWTPs would likely have been observed for the VH and not the MP area which is mainly under rainfed cultivation.

Another consideration is that the MDA-kb2 bioassays is approximately a 100-fold more sensitive towards potent AR agonists than potent GR agonists. The assay is therefore more likely to identify the activity of androgens rather than glucocorticoids in environmental samples (Conley *et al*., 2017). Although no AR and/or GR activation was found in this study, previous research has documented the androgen and glucocorticoid activity of environmental samples, including agricultural soil. Zhang *et al*. (2018) measured the total hormonal activities of agricultural soils using a battery of receptor bioassays, which included assessment of AR and GR activity. The authors found that 30% and 35% of the soil samples induced androgenic and glucocorticoid responses, respectively (Zhang *et al*., 2018). Stavreva *et al*. (2012) screened water samples collected across the Unites States of America for androgen and glucocorticoid activity. Results showed that of the tested water sources, 27% and 35% had glucocorticoid and androgen activity, respectively.

5.5.3 (Anti-)oestrogenic effects

(Anti-)oestrogenicity can be assessed using the human breast cancer cell line T47D-KB*luc* (Wilson *et al*., 2004). The T47D-KB*luc* cells respond to oestrogen-like compounds through the α-ER and β-ER meaning they are hormone-dependent (Yu *et al*., 2017). Xenoestrogens found in the environment bind to the ER with a 1000-fold lower affinity compared to endogenous oestrogens (De Falco *et al*., 2015). In South Africa,

research is especially focused on the oestrogenic effects of drinking- (Aneck-Hahn *et al*., 2009; Van Zijl *et al*., 2017; Aneck-Hahn *et al*., 2018), environmental- (Aneck-Hahn *et al*., 2008), and wastewater (Mahomed *et al*., 2008) with no studies investigating the endocrine disruptive effects of agricultural soil. This is problematic since many pesticide-active ingredients registered for use in South Africa cause oestrogenic effects, including DDT, chlorpyrifos, endosulfan, (Kojima *et al*., 2010), and glyphosate (Mesnage *et al*., 2017). Following rainfall and/or irrigation, these agrochemicals migrate towards aquatic environments, posing a threat to non-target biota. According to Schoenborn *et al*. (2015) washout of oestrogenic activity was highest during or right after heavy rainfall. One example of endocrine disruptive effects associated with exposure to environmental oestrogens is the feminisation of male fish (Kidd *et al*., 2007). The T47D-KB*luc* bioassay can be used to screen pesticide and environmental mixtures for (anti-)oestrogenic activities (Mutengwe *et al*., 2016) via the ER. This study used the T47D-KB*luc* bioassay to investigate whether the water-soluble compounds present in the maize and pecan soil contain any oestrogens and/or anti-oestrogens.

During the assessment of oestrogenicity, background activation of the ER was observed and as a result it was not possible to initially obtain a classical concentration-response curve for the reference compound, E_2, to which samples responses could be compared (these results are not shown in Chapter 4). However, this is not the first report of oestrogenic contamination in a tissue culture laboratory. Aneck-Hahn *et al*. (2005) experienced oestrogenic contamination during the establishment of the recombinant yeast screen bioassay used for the detection of oestrogenic activity. When comparing the water from four South African laboratories in terms of oestrogenic activity, it was evident that the oestrogenic contamination was attributed to the laboratory water sources (Aneck-Hahn *et al*., 2005). In addition, oestrogenic activity has been detected in South Africa's drinking water (Aneck-Hahn *et al*., 2009; Van Zijl *et al*., 2017). In our laboratory, drinking water from a tap is pre-filtered and then enters our in-house ELGA water purification system to obtain ultrapure deionised water (18.2 MΩ-cm). This could have been a potential source of the background activation experienced during the T47D-KB*luc* bioassay. Therefore, to reduce the oestrogenic contamination experienced several adjustments were made to the protocols used in our laboratory, especially those related to the use of our laboratory water (e.g., preparation of DPBS and nutrient medium). Prior to use, the ultrapure deionised water obtained from our in-house ELGA water purification system was filtered again, this time using a point of use biofilter—a positively charged nylon filter used to remove residual biologically active contaminants. When this did not reduce the background activation, the ultrapure deionised water from our in-house ELGA water purification system was replaced with high-purity water suitable for mass spectrometry.

Several types of plastic, plastic products, and chemical components of plastic, including bisphenol A and phthalates, are oestrogen active (Yang *et al*., 2011; Darbre, 2020). Exposure to UV light can cause leaching of chemicals from plastic (Klein *et al*., 2021). Therefore, specialised high-grade plastic consumables were used during routine culture maintenance as xenoestrogens are less likely to leach from these plastic

consumables (i.e., tissue culture dishes, centrifuge tubes, serological pipettes) into the nutrient medium of the cells. Moreover, where possible the plastic consumables (e.g., tissue culture dishes) were covered in tin foil to further prevent leaching of any oestrogenic-like compounds into the nutrient medium.

Unfortunately, none of these changes seemed to observably reduce the oestrogen background. The T47D-KB*luc* cells then received a background concentration of the know ER antagonist, ICI (0.04 ng/mL, ~IC_{20}). Moreover, the concentration range of the ER agonism reference compound, E_2, was prepared in nutrient medium also containing 0.04 ng/mL ICI. This is not normal practice for the assessment of agonistic responses using this reporter gene cell line and therefore responses obtained in this study can regrettably not be compared to results reported for ER agonism in literature. However, this co-incubation with the antagonist ICI was done in a final attempt to reduce the background oestrogenic activity experienced in our tissue culture laboratory and override any oestrogenic contamination from interfering with the assessment of ER activation of the samples (i.e., prevent false positive results). Although this situation was far from ideal, we hoped that we could at least compare sample sites to each other for evidence of ER agonism due to competitive binding for the ER between the ICI and any potential oestrogens present in the samples. Although the use of an ER antagonist reduced the activation background enough so that sample responses could be evaluated, $\%E_2$ Max values for the lowest three E_2 concentrations, BC, and SC below 20% could not be obtained. During this study, the source of the oestrogenic contamination could not be identified. This warrants further investigation before any future T47D-KB*luc* bioassays can be performed in this laboratory.

Most studies express samples responses in terms of the reference compound as biological equivalents (e.g., E_2-EQ) (Xiao *et al*., 2006; Weiss *et al*., 2009). However, this was not possible in the present study as the sample extracts did not give a classical agonism concentration response curve (**Figure 23**). Consequently, the luciferase activity of samples responses was expressed as a FC compared to the SC. None of the MP samples showed oestrogenic activity (**Table 12**; **Table 19**; **Figure 24**). The findings of the present study indicate that only three VH samples (M13, P2, and P3) caused statistically significant oestrogenic responses (FC > 1) (**Figure 24**). Although a fold induction > 1.5 has been reported as the cut-off value for ER activation in literature, these authors (Lundqvist and colleagues) exposed cells to concentrated water samples (Lundqvist *et al*., 2019). Soil extracts in the current study were purposefully not concentrated to mimic environmental conditions as far as possible. In addition, the response of environmental mixtures in *in vitro* bioassays are usually very low. Based on this, a FC > 1 was considered as a suitable cut-off value for ER agonism in the present study.

The three VH samples (M13, P2, and P3) that showed ER agonism (FC > 1) (**Figure 24**), all had quantifiable levels of atrazine and dicamba (**Table 16**). Although atrazine was present in the soil samples of the present study, studies have shown that atrazine is not an ER agonist (Connor *et al*., 1996) and that the endocrine disruptive effects induced by atrazine are not due to receptor binding but rather ER-independent interactions such as influencing the activity of enzymes involved the biosynthesis of steroid

hormones. Holloway *et al*. (2008) evidenced that atrazine increases aromatase—the enzyme responsible for the conversion of androgens into oestrogens (Gea *et al*., 2022)—in human granulosa cells, while Roberge *et al*. (2004) showed that atrazine does not interact with the α-ER or β-ER but competitively inhibits phosphodiesterase. This is problematic as phosphodiesterase inhibition can increase the expression of aromatase by mRNA (Roberge *et al*., 2004). The inability of atrazine to act as an ER agonist has also been thoroughly reviewed by Eldridge *et al*. (2008). Although dicamba was also quantified in M13, P2, and P3, no comparable literature could be found about the ER agonistic potential of dicamba. Apart from atrazine and dicamba, other agrochemicals and/or their metabolites were also detected in the soil samples (**Tables S13–S14**). Some of which have been evidenced to induce oestrogenic responses via the ER, including bromophos-ethyl, endosulfan sulfate, fenarimol, glyphosate, isofenphos, and methiocarb (Andersen et al., 2002; Kojima *et al*., 2004; Mesnage *et al*., 2017; Gea *et al*., 2022).

The highest fold change of ER agonistic activity was almost 1.8-fold compared to the SC and was induced by M13 (**Figure 24**). Based on the qualitative chemical analysis glyphosate was detected in M13 (Table S14). Glyphosate-based herbicides (GBHs) are the most used herbicide class worldwide (Horn *et al*., 2019). In South Africa, approximately 66% of the country's glyphosate is applied to maize crops (Dabrowski *et al*., 2014). Since the MP is the country's largest maize producer in terms of average yield (1 000 t/ha) (Grain SA, 2021), the area receives a considerable input of GBHs (Dabrowski, 2015b; Dabrowski, 2015c). Some authors have reported that glyphosate as the active ingredient causes ER agonism. Mesnage and colleagues (2017) found that glyphosate activated the α-ER in T47D-KB*luc* cells at high concentrations. Despite this, it was concluded that the ER agonistic effects induced by glyphosate were likely due to a ligand-independent mechanism (Mesnage *et al*., 2017). Thongprakaisang *et al*. (2013) demonstrated that environmentally relevant concentrations of glyphosate induced activation of the ERE in T47D-KB*luc* cells and altered the expression of α-ER and β-ER. Although these studies assessed the effects of pure glyphosate it is important to also consider the endocrine disruptive effects induced by pesticide formulations as these effects are more representative of what may occur when chemical mixtures end up in the environment.

Furthermore, since M13 showed the highest ER agonism (**Figure 24**), it was suggested that pesticides present in M13 but absent in P2 and P3 may be responsible for the observed effect. Imidacloprid was the only pesticide quantified in M13 but not in P2 and P3 (**Table 16**). Since previous literature has reported ER agonistic effects following imidacloprid exposure, this could explain why P2 and P3 showed slightly lower ER agonism in comparison to M13 (**Figure 24**). Zhang *et al*. (2020) found that imidacloprid exposure increased the relative luciferase activity in a dose-dependent manner in MELN cells (human breast cancer cells that contain a luciferase reporter gene and ERE) compared to the reference compound, E_2. This indicated oestrogenic activity. Contrary to the findings of Zhang *et al*. (2020), Mesnage *et al*. (2018) did not find any oestrogenic activity in the hormone-dependent, human breast cancer cell line, MCF-7,

following exposure to seven neonicotinoid active ingredients, including imidacloprid (1–300 mg/L; 6 days). However, Mesnage *et al*. (2018) only used a cell proliferation assay to determine oestrogenic activity and not a reporter gene bioassay which directly assesses receptor binding as in the case of Zhang *et al*. (2020) and the present study.

Apart from the potential effect of pesticides present in the soil sample, an additional explanation for the observed ER agonistic activity in the present study, is the presence of natural plant derived compounds which are structurally and functionally related to human oestrogens (i.e., phytoestrogens). Phytoestrogens can bind to and activate both the α-ER and β-ER (Celma *et al*., 2021; Sim *et al*., 2022). Several fruit tree, grain, and vegetable crops produce phytoestrogens, including apples, avocados, beans, maize, pecans, and soybean (Mostrom & Evans, 2011) and consequently these compounds have been detected in agricultural soils. The concentration of phytoestrogens in receiving water bodies is also higher during runoff conditions (Hama *et al*., 2021). In South Africa, soybean is often used in rotation with maize and the oestrogenicity of soybean has been reported (Morgan *et al*., 2014). Since samples from one maize field (M13) and two pecan orchards (P2 and P3) exhibited ER agonism, the phytoestrogens produced by these respective crops (i.e., maize and pecans) and crops used in rotation (i.e., soybean) could be a potential source of the oestrogenicity. High concentrations of androgens can bind to and activate the ER (Christiaens *et al*., 2005). Yet, since none of the samples showed androgenicity this was likely not the cause of the observed ER agonism. Xioa *et al*. (2006) used a battery of *in vitro* bioassay to evaluate the toxicological effects of surface soil from Tianjin, China and the authors attribute the high oestrogenic activity in some samples to effluent from upstream areas (Xiao *et al*., 2006).

For ER antagonism, E_2 (5.4 pg/mL, EC_{80}) was used as the agonist. In the present study, only one sample (M13) caused ER antagonism which may be attributed to the presence of anti-oestrogens and/or matrix effects (Li *et al*., 2015; Schoenborn *et al*., 2015). Previous studies have detected anti-oestrogens in soil (Li *et al*., 2015; Zhang *et al*., 2018) and reports of pesticides with anti-oestrogenic activity are found in literature (Sohoni & Sumpter, 1998; Okubo *et al*., 2004). What is more, matrix effects can modulate antagonistic responses in environmental samples, such as soil (Schoenborn *et al*., 2015), influencing the outcome of the bioassay. Matrix effects refer to differential biological responses elicited by anthropogenic pollutants and naturally occurring compounds present in the sample matrix (i.e., soil in the case of this study) (Abbas *et al*., 2019). Natural compounds such as humic acid and colloidal organic carbon are present in soil and can affect the results obtained for soil during *in vitro* reporter gene bioassays (Li *et al*., 2015). Humic substances are formed when organic matter decomposes and previous studies have evidenced the anti-oestrogenicity of humic acids (Janošek *et al*., 2007). Colloids—particles that are 1–1 000 nm in size—are ubiquitous in all environments and the majority of the organic carbon in soil is present in the form of colloids (Yan *et al*., 2018). Colloidal organic carbon is the most biologically active form of soil due to its large surface area (Yan *et al*., 2018). Consequently, the presence of contaminants (e.g., oestrogen-like compounds) in soil samples can adsorb to colloids, preventing the oestrogen-like compounds from binding to the ER and

inhibiting oestrogenic activity (Li *et al*., 2015). Moreover, dissolved organic carbon has been shown to interfere with the ER reporter gene bioassays by adsorbing to E_2 during the assessment of ER antagonism and preventing its bioavailability to cells. To limit the adsorption of dissolved organic carbon E_2 during the assessment of ER antagonism, an EC_{80} agonist concentration was used (Neale *et al*., 2015). Overall, the total organic carbon content of the MP and VH samples was low to moderately low (**Table 7** and **Table 19**). Although it is improbable that colloidal and dissolved organic carbon in the samples affected the outcome of the T47D-KB*luc* bioassay for ER antagonism, it cannot be ruled out.

In the present study, slightly more samples had agonistic oestrogenic effects (16%; **Figure 24**) compared to those with anti-oestrogenic effects (5%; **Figure 26**). These findings are in line with Zhang *et al*. (2018) who reported that 79% of the total agricultural soil samples evaluated for (anti-)oestrogenic activity showed ER agonistic potential. Only 16% exhibited ER antagonism (Zhang *et al*., 2018). In opposition, when investigating the ER activities in soil sampled along the Second Songhua River in China, Li *et al*. (2015) found that anti-oestrogenicity was more prevalent than oestrogenicity. Furthermore, it was concluded that the hydrophilic fraction (polar compounds) was the key fraction involved in the increasing anti-oestrogenic activity. Maize 13 was the only sample that showed ER antagonism (**Table 13**; **Table 19**; **Figure 26**). As previously mentioned, glyphosate was detected in M13 and apart from showing ER agonism, ER antagonism has been evidenced for certain glyphosate formulations. Gasnier *et al*. (2009) assessed anti-oestrogenicity in human liver HepG2 cells (which were transiently transfected with ERE-TK-luciferase, α-ER, and β-ER) following exposure to four glyphosate formulations, including Roundup Express®, Bioforce®, Grands Travaux®, and Grands Travaux plus®. All four glyphosate formulations caused ER antagonism (Gasnier *et al*., 2009). However, the endocrine disruptive potential of glyphosate and GBHs is a controversial topic due to conflicting results in the literature as reviewed by De Araújo-Ramos and colleagues (2021). Consequently, this warrants further investigation.

Interestingly, M13 was also the only sample that contained water-soluble compounds with binding affinities for both the AR (anti-androgenic activity; **Figure 22** and **Table 19Table 19**) and ER [(anti-)oestrogenic activity; **Figure 24** and **Table 19**], supporting potential pleiotropic effects (Kojima *et al*., 2004; Kojima *et al*., 2010). Pleiotropy is when a compound can act via several mechanisms and as a result has multiple endocrine bioactivities (Sohoni & Sumpter, 1998). This is a common feature of many pesticides and has been reported for fenarimol, isofenphos, and methiocarb (Kojima *et al*., 2010). All of which are registered pesticides in South Africa (Quinn *et al*., 2011; Dabrowski, 2015b; Dabrowski, 2015c) and were also detected in some of the soil samples of the present study (**Tables S13–S14**). It is important to note that although the hormonal activities of environmental samples are usually low, their ability to act via several mechanisms might enhance their overall biological effect due to the interconnectedness of the affected pathways (Andersen *et al*., 2002). Based on the overall results obtained for endocrine disruptive effects, it is reasonable to assume that the anti-androgenic and (anti-)oestrogenic activity caused by the samples in the present study can be attributed to the agricultural practices associated with the sampling locations.

The two sampling locations (MP and VH) were selected for their high input of pesticides, especially herbicides. No weeds were present at most of the sampling locations (**Table S1**) for which samples showed endocrine disruptive effects (M1, M4, M7, M8, M9, M10, M13, P3, and P4; **Table 19**), indicating that herbicides are most certainly being applied. This was also evidenced by the data obtained for the qualitative chemicals analysis which shows that overall herbicides (37%) and insecticides (36%) were the most representative classes of agrochemicals (**Figure 36**; **Figure 37**; **Tables S13–S14**). The only exception was P2, which showed ER agonism (**Figure 24**) although the pecan orchard was covered in weeds (**Table S1**). During sampling there were no cattle present at any of the sampling locations (**Table S1**) for which the samples showed endocrine disruptive effects (M1, M4, M7, M8, M9, M10, M13, P2, P3, and P4) (**Table 19**). However, the anti-androgenic and (anti-)oestrogenic activity may partly originate from nearby livestock (Lundqvist *et al*., 2019). Livestock, such as cattle, are a source of both natural (e.g., testosterone, E_2) and synthetic (e.g., methyltestosterone, trenbolone acetate) steroid hormones. The former is naturally produced by farm animals while the latter is often used as growth regulators that increase feed efficiency, directly promoting muscle strength and enhancing the meat quantity in farm animals (Weiss *et al*., 2009). These growth hormones end up in animal manure and when applied as fertilisers, it represents a direct route of exposure to the soil (Casey *et al*., 2004). This is worrisome as steroid hormones originating from animal manure can persist in composting after 171 days, with only a 40.4% removal rate and have been detected in crops (roots of Chinese cabbage) (Zhang *et al*., 2019).

Casey *et al*. (2004) investigated the sorption, transformation, and mobility of testosterone in agricultural soils and found that testosterone was degraded more readily compared to E_2. Yet, testosterone had a greater potential to migrate in the soil, increasing its potential to run-off. It has been demonstrated that oestrogenic activity in water samples from watersheds are higher when samples were collected during runoff conditions following the application manure as a fertiliser. Runoff conditions following manure application increase oestrogenic activity in receiving watersheds (Alvarez *et al*., 2013). When synthetic hormones persist in soil for several days it would not necessarily influence surface waters (e.g., rivers, dams, and streams). Nonetheless, if the hormones escape the upper soil horizon it increases the potential for subsurface water contamination, putting groundwater sources at risk (Casey *et al*., 2004).

5.6 Oxidative stress and damage responses in human duodenum cells

5.6.1 Reactive oxygen species production, superoxide dismutase content, and catalase activity

Reactive oxygen species are endogenously produced as by-products of cellular metabolism and energy production in the mitochondria (Ray *et al*., 2012). When present in physiological concentrations ROS have important functions (Jabłońska-Trypuć *et al*., 2017; Pisoschi & Pop, 2015; Ventura *et al*., 2015) and these ROS are neutralised by the intracellular antioxidant defence system (Gargouri *et al*., 2020). However, when ROS production surpasses the ability of the antioxidant defence system to neutralise excess ROS it causes oxidative stress (Archibong *et al*., 2018; Burella *et al*., 2018; Jabłońska-Trypuć *et al*., 2017). Prolonged

oxidative stress leads to oxidative damage where ROS interact with macromolecules including proteins, lipids, and DNA (Huang *et al*., 2020a). The antioxidant enzymes, SOD and CAT represent the first line of defence against ROS. Superoxide dismutase is the enzyme responsible for catalysing the conversion of superoxide radicals (one type of ROS) into H_2O_2 and O_2 (Ventura *et al*., 2015). Catalase is responsible for further detoxification of the H_2O_2 by catalysing its decomposition into H_2O and O_2 (Chance *et al*., 1948). Overall, the neutralisation of ROS and detoxification of pesticides initially increases SOD and CAT activity, while reduced activity could indicate that these protective enzymes are overwhelmed and can no longer mitigate the damage caused by ROS generation (Basopo *et al*., 2014; Mossa *et al*., 2015; Topal *et al*., 2017).

If humans or wildlife ingest environmental water which has been contaminated with agrochemicals, the digestive tract is susceptible to direct exposure (Ilboudo *et al*., 2014). Consequently, the intestines represent the most common exposure route following consumption. Therefore, intestinal cells (HuTu-80) were used for the assessment of various oxidative stress endpoints. Although the human duodenal adenocarcinoma cell line (HuTu-80) was initially used in cancer research (Schmidt *et al*., 1977), these cells have also been used to investigate oxidative stress-induced apoptosis (Pereira Moreira *et al*., 2009). After xenobiotic parent compounds and metabolites have been absorbed by the GIT, these compounds enter the liver to be metabolised and undergo biotransformation reactions. *In vitro* cell line models originating from the liver, retain all the biotransformation enzymes of the liver and are relevant models for hepatoxicity in whole organisms (Huang *et al*., 2020a). Other authors have demonstrated the usefulness of using liver cells when investigating oxidative stress endpoints (Žunec *et al*., 2016; Kašuba *et al*., 2017). The H4IIE-*luc* rat hepatoma cell line was therefore used to assess oxidative stress responses likely to occur in the liver. Due to the interconnectedness of ROS production and the detoxification actions of SOD and CAT, the results of these three oxidative stress endpoints are discussed collectively.

In the present study, many of the samples caused a redox imbalance in the human intestinal cells by changing the level and/or activity of the first line of defence antioxidant enzymes (i.e., SOD and CAT) (**Table 19**). This is worrisome as any change in this oxidative balance within cells may alter the integrity of the cells (Gomez *et al*., 2020). Overall, 68%, 74%, and 63% of all the samples (MP and VH) elicited a significant response for ROS production, SOD content and CAT activity, respectively (**Table 19**). The high levels of ROS reported in the HuTu-80 cells for several of the MP samples (M1–M5) were comparable to and higher than the response in the cells after exposure to the positive control (cells stimulated with H_2O_2). This indicates that there are water-soluble compounds present in the MP samples that act similarly to H_2O_2. However, it is difficult to identify the origin of the elevated ROS levels, since different agrochemicals were present in each of these five soil samples (**Table 16**). Increased ROS production is damaging to cells (Huang *et al*., 2020a) and other studies have reported elevated levels of ROS in human neuroblastoma (SK-N-SH) and first trimester trophoblast (HTR-8/SVneo) cells following 24 hours of exposure to pesticide active ingredients and/or commercial formulations (Gargouri *et al*., 2020; Gomez *et al*., 2020).

A noteworthy observation is that M1 had the highest concentration of imidacloprid (96.9 ng/g; **Table 16**) which could have potentially contributed to increased ROS. Ge *et al*. (2015) found that exposure to 1.5 and 5 mg/L of imidacloprid for 14, 21, and 28 days significantly increased ROS production in zebrafish (*Danio rerio*). Samples from M1–M5 only slightly increased the SOD content compared to the blank control (**Figure 29**; **Table 19**; **Table S6**), suggesting that the enzyme did not entirely detoxify the superoxide anions produced by the cells, and little or no H_2O_2 was produced. As a result, the ROS levels remained significantly high. Low concentrations of H_2O_2 would explain why the CAT activity of M1–M5 was lower compared to the untreated blank control cells (Bebe & Panemangalore, 2003)—there was not enough substrate available to increase CAT enzymatic activities. However, this reduction in CAT activity was not statistically significant. Another potential explanation for the accumulation of ROS is the depletion of GSH, the main antioxidant in living organisms. The reduced form of glutathione neutralises ROS via electron donation. Exposure to xenobiotics can also deplete cellular GSH, increasing ROS generation (Gargouri *et al*., 2020). One example is glyphosate (detected in M2; **Tables S13–S14**) which rapidly decreased GSH in the human prostate cell line WPM-Y.1 (Abdel-Halim & Osman, 2020). Since GSH content in the cells was not measured in this study, the effects of potential GSH depletion cannot be ruled out.

Although the highest level of 2,4-D (832.4 ng/g) was quantified in M6 (**Table 16**), the statistically significant reduction in ROS production in the HuTu-80 cells after exposure to M6 indicates that the antioxidant defence system was able to neutralise the ROS and consequently SOD content and CAT activity did not show any significant response compared to the blank control (**Figure 31**; **Table 19**). The most logical explanation is that after the excess ROS was neutralised, the two enzymes returned to a balanced state (i.e., no significant increase or decrease in activity). Contrary to these results Hattab *et al*. (2015) reported that 2,4-D exposure in *Eisenia andrei* not only affected the earthworm physiology but also induced oxidative stress by significantly altering the activity of antioxidant enzymes (SOD, CAT, and GST). Another author corroborated the findings that 2,4-D toxicity in non-target organisms may be related to the prevalence of oxidative stress (Ozcan Oruc *et al*., 2004). Alternatively, natural compounds with antioxidative potential may be present in the soil. This is plausible since measurable quantities of antioxidants have been extracted from soil representing different types of land-use, including arable cropping, in the United Kingdom (Rimmer & Smith, 2009). Moreover, certain soil fungi naturally produce antioxidants (Skanda & Vijayakumar, 2021).

The fluorescence intensity after exposure to M7, M10 and M11 also significantly decreased (**Figure 27**; **Table 19**; **Table S4**). As the fluorescence is proportional to the amount of ROS produced (Martínez *et al*., 2020), this indicates a reduction in the level of ROS. Superoxide radicals may have induced SOD activity, elevating the levels of the enzyme to convert superoxide radicals into H_2O_2—reducing overall ROS. Consequently, CAT activity of all three of these samples also increased significantly in response to the increased H_2O_2 following SOD activity (Farombi *et al*., 2008; Basopo & Muzvidziwa, 2020). Catalase detoxified the H_2O_2 by converting it into water and oxygen (Chance, 1948). This is in accordance with the

literature because exposure to environmental pollutants usually increases SOD and CAT activity (Farombi *et al*., 2008). Taken together, these results show that the antioxidant defence system of the HuTu-80 cells was able to counterbalance the oxidative stress (in the form of ROS) induced by these samples, even for M11 which had the highest pesticide load of all sampling locations (ΣPesticide = 8 108.9 ng/g) (**Table 16**).

Exposure to M8 and P3 significantly reduced both the ROS and SOD content yet increased the CAT activity (**Table 19**). Although M8 had the highest atrazine concentration (208.6 ng/g; **Table 16**), it seems the SOD was able to detoxify any resulting $O_2^{\bullet-}$ (reducing overall ROS), but the enzymatic activity was diminished by this. It is also possible that elevated levels of H_2O_2 inhibited the SOD activity as reported by Ventura *et al*. (2015). Excess H_2O_2 may still have been present in the cells and CAT catalysed the decomposition of the remaining H_2O_2 into water and oxygen, explaining the significant increase in CAT activity (Chance *et al*., 1948). Even though exposure to M9 resulted in the reduction of ROS production, there was no notable change in SOD content when compared to the blank control (untreated cells) and it can be assumed that superoxide radicals were not neutralised. The subsequent decrease in CAT activity can therefore be attributed to the flux of $O_2^{\bullet-}$ which are known to inhibit CAT activity (El-Demerdash, 2011; Ansari & Ansari, 2014) by affecting the heme group located in the active site of the enzyme (Astiz *et al*., 2009).

All the VH samples that did not significantly affect ROS production (M12–M15, P2, and P4; **Figure 27**; **Table 19**; **Table S4**), showed a significant decrease in SOD content (**Figure 29**; **Table 19**; **Table S6**). The low levels of SOD were in all probability due to the utilisation of the enzyme which eventually led to SOD inactivation or depletion (Akbel *et al*., 2018). Nevertheless, all these samples significantly increased CAT activity (**Figure 31**; **Table 19; Table S8**), potentially due to the presence of H_2O_2 in the HuTu-8 cells which was generated before the SOD was depleted. Resultantly, CAT activity significantly increased to neutralise the produced H_2O_2. Notably, carbosulfan was present in all the aforementioned VH samples (**Tables S13-S14**), with the literature showing that carbosulfan can significantly alter the activities of antioxidative enzymes such as CAT and SOD in the liver of rainbow trout (*Oncorhynchus mykiss*) (Capkin & Altinok, 2013) and Wistar rats (El-Bini Dhouib *et al*., 2015).

Furthermore, compounds can induce oxidative stress by interacting with an organism's ROS generation system, leading to an overproduction of $O_2^{\bullet-}$ (Huang *et al*., 2020a). This was the case for one of the VH samples (P1) which significantly increased ROS generation (**Figure 27**; **Table 19**; **Table S4**). According to Da Silva Barreto *et al*. (2020), an increase in ROS levels indicates a redox dysfunction. Pecan 1 caused the SOD to be significantly downregulated in the HuTu-80 cells (**Figure 29**; **Table 19**; **Table S6**), but CAT activity was unaltered (**Figure 31**; **Table 19**; **Table S8**). This could be attributed to the fact that SOD and CAT are the primary and secondary lines of defence against xenobiotics, respectively (Huang *et al*., 2020a). Any superoxide radicals produced after exposure to P1 were initially converted into H_2O_2 and oxygen by SOD, decreasing the amount of enzyme present in the cell. However, it is important to note that SOD activity is dependent on the cellular oxidative environment, as well as SOD gene regulation by cytokines

(Bebe & Panemangalore, 2003). Since the ROS remained high, it is possible that SOD could no longer detoxify the superoxide radicals due to their overproduction, and reduced SOD gene regulation. This led to the depletion of the enzyme and H_2O_2 was no longer produced. Subsequently, CAT activity remained unchanged. These results are consistent with the findings of Huang *et al*. (2020a) who investigated the effect of the herbicide acetochlor on several oxidative stress endpoints (ROS, SOD, CAT and GSH) in HepG2 cells. Acetochlor was present in some of the soil samples (**Table S13**).

5.7 Oxidative stress responses in the rat hepatoma cell line

5.7.1 Reactive oxygen species production, superoxide dismutase content, and catalase activity

Comparable results to the HuTu-80 cells (**Table 19**) were obtained where 68% of the samples elicited a significant response for ROS production in the H4IIE-*luc* cells (**Figure 28**; **Table 19**; **Table S5**). However only 37% and 26% of the samples showed a significant response for SOD content (**Figure 30**; **Table 19**; **Table S7**) and CAT activity (**Figure 32**; **Table 19**; **Table S9**), respectively. This is likely attributed to the primary function of the liver which is detoxification. Due to this, the antioxidant defence system of the liver is also more robust compared to that of other organs (Astiz *et al*., 2009). The H4IIE-*luc* rat liver hepatoma cells were able to detoxify the xenobiotics present in the samples. Furthermore, although the HuTu-80 cells were stimulated with 3.5 ng/mL H_2O_2 to induce ROS production, a higher concentration of H_2O_2 (14.2 ng/mL) was necessary to obtain a similar response in the H4IIE-*luc* cells. This demonstrates that the liver cells can withstand exposure to a higher concentration of xenobiotics and requires significant intoxication before the antioxidant defences are initiated. This is supported by Astiz *et al*. (2009) when comparing the oxidative stress responses in rat liver and testes following five weeks of exposure to dimethoate, glyphosate and zineb. The rat testes were more sensitive towards the pesticides in comparison to the liver.

When comparing the oxidative stress responses between the HuTu-80 and H4IIE-*luc* cell lines, differences were observed for most of the samples. This discrepancy is likely attributed to the fact that these cell lines originate from different organ tissues and therefore have different basal levels (response in untreated blank control cells) of ROS, SOD content and CAT activity (Ventura *et al*., 2015). Astiz *et al*. (2009) reported differences in antioxidant enzyme activities in the liver and testes of rats. These differences were also attributed to the different physiological competencies of the respective organs. Of the MP samples, only M1, M5 and M7 caused a significant increase in ROS production, while M4, M8, and M9 reduced the amount of ROS (**Figure 28**; **Table 19**; **Table S5**). Responses elicited by the VH samples followed a similar trend with four samples increasing (M12, M13, M14, and P2) and three decreasing (M15, P3, and P4) ROS production (**Figure 28**; **Table 19**; **Table S5**).

Apart from increasing ROS production, SOD content and CAT activity were not significantly affected after exposure to M1 (**Table 19**). This means that the amount of ROS most probably overwhelmed and depleted

the antioxidant defence system, reducing these antioxidant endpoints (i.e., SOD and CAT) (Huang *et al*., 2020a). These findings agree with Ventura *et al*. (2015) who reported that SOD and CAT enzymatic activities remained unchanged after two human breast cancer cell lines, MCF-7 and MDA-MB-231, were exposed to the insecticide, chlorpyrifos (0.05 µM; 24 hours). Even though chlorpyrifos was not present in M1, several other OPs were detected in the sample which may have the same or similar oxidative effects as chlorpyrifos, including bromophos-ethyl, diazinon, ethoprophos, fenamiphos, isofenophos, and tetrachlorvinphos (**Tables S13–S14**). Although M2 and M3 showed no response for ROS and CAT activity, M2 slightly increased SOD content (**Table 19**). Any excess ROS present was likely detoxified quickly by SOD activity. Maize 5 and M7, on the other hand, caused a statistically and practically significant increase in SOD content in the H4IIE-*luc* cells but no response in CAT activity (**Table 19**). Yet, since ROS remained slightly higher compared to the blank control, the conversion of ROS into H_2O_2 and oxygen by SOD was perhaps still in progress, and therefore the concentration of intracellular H_2O_2 was low (Bebe & Panemangalore, 2003). Consequently, CAT activity was not significantly different in comparison to the blank control cells. The inactivity of CAT due to low levels of H_2O_2 is confirmed by Bebe and Panemangalore (2003) who investigated the effect of endosulfan and chlorpyrifos on the endogenous antioxidants in the liver, lungs, and erythrocytes of rats. Nonetheless, SOD only converts superoxide radicals into H_2O_2. Therefore, the overall ROS can remain high due to the presence of other reactive species (e.g., hydroxyl or peroxyl groups) (Gargouri *et al*., 2020).

After exposure to M4 and M8, SOD in the H4IIE-*luc* cells increased to reduce the amount of ROS inside the cells. The SOD content was only slightly higher compared to the blank control cells and the antioxidant defence system was able to neutralise the ROS (**Figure 30**; **Table 19**; **Table S7**). Topal *et al*. (2017) also ascribed elevated levels of SOD activity in the brain of rainbow trout as a response against oxidative stress following imidacloprid exposure. Yet, the amount of H_2O_2 produced following M4 and M8 exposure was not necessarily enough to significantly increase CAT activity (**Table 19**). The ROS level after exposure to M6 may have been elevated but was neutralised by SOD, increasing the enzyme's content (**Figure 30**; **Table 19**; **Table S7**), and restoring redox balance within the cells. Exposure to M9 caused a reduction in ROS generation, but the SOD content remained unchanged (**Table 19**). On the other hand, CAT activity significantly increased, suggesting increased levels of superoxide anions and/or H_2O_2 (Topal *et al*., 2017) (**Figure 32**; **Table 19**; **Table S9**). Maize 10 did not affect the ROS, SOD content or CAT activity in the H4IIE-*luc* cells (**Table 19**) indicating that either the samples do not contain compounds that cause oxidative stress, or the liver cells were able to mitigate the effects of low levels of pesticides and prevent oxidative stress. This is a plausible explanation since M10 had the second lowest pesticide load of all the samples (ΣPesticides = 32.8 ng/g; **Table 16**). These results agree with a study by Kašuba *et al*. (2017) who investigated the oxidative stress responses elicited in HepG2 cells following exposure to glyphosate concentrations likely to be encountered in occupational and residential exposure. The fact that no significant change in ROS was detected was also ascribed to the efficient removal of ROS by the antioxidant defence system (Kašuba *et al*., 2017). The significant reduction in SOD content for M11 (**Figure 30**; **Table 19**;

Table S7) indicated the amount of enzyme decreased or was depleted due to the rapid or continuous conversion of superoxide radicals into H_2O_2 (Kapoor *et al*., 2010; Mossa *et al*., 2015). Redox balance was restored within the H4IIE-*luc* cells after exposure to M11 as CAT activity remained unaltered (**Figure 32**; **Table 19**; **Table S9**).

None of the four VH samples which significantly increased the level of ROS (M12, M13, M14, and P2; **Figure 28**; **Table 19**; **Table S5**) affected the SOD content (**Figure 30**; **Table 19**; **Table S7**). The intracellular ROS may have overwhelmed or depleted the antioxidant defence system due to enzyme and/or substrate depletion (Huang *et al*., 2020a), or caused direct enzyme inhibition by impairing the functional groups in the enzyme's structure (JanakiDevi *et al*., 2013). If some H_2O_2 was still present in the cells it would explain the slight increase in CAT activity for M12 and M14 as opposed to M13 and P2 (**Figure 32**; **Table 19**; **Table S9**). For P1, ROS and SOD content remained unchanged while CAT activity significantly increased (**Table 19**) likely due to an influx of H_2O_2 (Farombi *et al*., 2008; Basopo & Muzvidziwa, 2020). The antioxidant defence system of the H4IIE-*luc* cells effectively reduced ROS production after exposure to M15 and P4 (**Figure 28**, **Table 19**; **Table S5**). Consequently, neither SOD content nor CAT activity showed any significant responses (**Table 19**). On the contrary, soil samples (M15, P3, and P4; **Table 19**) might contain compounds with antioxidative potential (Rimmer & Smith, 2009; Skanda & Vijayakumar, 2021) due to a reduction in ROS. Overall, the results show that water-soluble compounds present in agricultural soil caused significant oxidative stress *in vitro* in both human intestinal and rat liver cells. However, the H4IIE-*luc* cells were more capable of counterbalancing the induced oxidative stress compared to the HuTu-80 cells (**Table 19**).

5.8 Peroxidative damage to cellular lipid components

After prolonged exposure to environmental pollutants, an organism's antioxidant defence system can become overwhelmed. This leads to oxidative damage when ROS interact with important macromolecules (i.e., lipids, proteins, and nucleic acids). When ROS interact with lipid components present in the cellular membrane of an organism it causes oxidative degradation, known as lipid peroxidation. The presence of these lipid peroxides can cause the loss of cell membrane structure and function (Romero *et al*., 2012). In view of this study's results, none of the samples induced the production of the lipid peroxidation by-product, MDA, in either of the two cell lines (HuTu-80 and H4IIE-*luc*) at the concentration (83 mg/mL) investigated (**Figure 33**; **Figure 34**; **Table 19**; **Tables S10–S11**). These results indicate that no oxidation of lipids in the cellular membranes occurred. This is also further supported by the conversion of TBA into MDA in the TMP standards (**Figure 33**; **Figure 34**; **Table 19**; **Tables S10–S11**). These findings and the presence of imidacloprid in some of the soil samples (**Table 16**) in the current study are consistent with a previous study. Želježić *et al*. (2016) reported that acute 24-hour exposure to three different pesticides (α-cypermethrin, chlorpyrifos, and imidacloprid) did not significantly affect LPO in the human liver carcinoma cell line, HepG2.

There are several plausible reasons why LPO did not occur in the HuTu-80 and H4IIE-*luc* cells after exposure to the samples. Since SOD and CAT play an important role in preventing the formation of lipid peroxides (Gargouri *et al*., 2020) and based on the results obtained during the SOD and CAT bioassays, it is evident that the antioxidant defence system in both the HuTu-80 and H4IIE-*luc* cells were active even though glyphosate was detected in some of the soil sample (**Tables S13–S14**). This was evidenced by Ferreira *et al*. (2010) during the assessment of oxidative stress endpoints measured in the liver of the South American catfish (*Rhamdia quelen*) following glyphosate exposure.

Oxidative stress endpoints are concentration-and time-dependent (Ansari & Ansari, 2014). As a result, the exposure concentration and period may not have been sufficient for the specific cell lines under investigation to cause lipid peroxidation. Oxidative damage usually occurs after prolonged exposure and when the antioxidant system has been completely overwhelmed by oxidative stress. Since the HuTu-80 and H4IIE-*luc* cells were only exposed to the samples for 24 hours, it was perhaps not long enough for oxidative damage to occur. Due to the role of the intestines and liver in the detoxification of xenobiotics, intestinal and liver cells may require longer periods of exposure to environmental pollutants before LPO is induced. Any ROS produced were probably already detoxified before they could interact with lipid components present in the cellular membranes. Ilboudo *et al*. (2014) reported LPO after the human intestinal cell line Caco-2 was exposed to 25 and 100 µM of several pesticides, including deltamethrin, fenitrothion, and fipronil, for 48 hours. Furthermore, Weis *et al*. (2021) provided evidence of LPO in BV-2 murine microglial cells after 96 hours of exposure to chlorpyrifos (100 and 300 µM). None of these abovementioned pesticides were identified in any of the soil samples during the present study (**Tables S13–S14**), perhaps explaining why LPO did not occur. It is possible that the water-soluble compounds present in the sample did not interact with the lipid components of the cells but rather scavenged other macromolecules, such as proteins and/or nucleic acids (Kašuba *et al*., 2017). This would also explain the absence of any LPO, and since protein carbonylation and DNA damage were not assessed in this study, it cannot be ruled out.

Other authors have reported contradictory findings. Exposure to deltamethrin (10 µM) significantly increased the production of lipid peroxides in human dopaminergic neuroblastoma cells (SH-SY5Y) after 8 hours of exposure (Romero *et al*., 2012). A significant increase in MDA levels was reported by Gomez *et al*. (2020) in human first-trimester trophoblast cells after only 4 hours of exposure to a commercial acetamiprid formulation. Moreover, 24 hours of exposure to the OP pesticides, diazinon, significantly increased the level of MDA in human intestinal cells (HCT116) in a dose-dependent manner (Boussabbeh *et al*., 2016). However, only diazinon was detected in the soil samples during the present study but showed low abundance (< 3 000; **Tables S13–S14**).

In addition, the TBARS assay is restricted by low sensitivity since it has a limit of detection of approximately 1.1 µM MDA. This could affect the results obtained if cells produce very low levels of MDA

after exposure to samples (Tabernilla *et al*., 2021). Furthermore, the number of cells used during the TBARS assay may not have been enough for the cells to produce quantifiable amounts of MDA. In this study, a seeding density of 80 000 cells/mL was used for all oxidative stress and damage bioassays. However, this seeding density might not have been high enough to elicit a measurable response for specifically LPO. Different seeding densities have been reported in the literature for the TBARS assay: 100 000 cells/mL (Kašuba *et al*., 2017) for HepG2 cells; 125 000 cells/mL for Caco-2 cells (Ilboudo *et al*., 2014); and 150 000 cells/mL for HEp-2 cells (Coalova *et al*., 2014). As in the case of this study, most authors seeded cells in 24-well microplates during oxidative stress (Chaufan *et al*., 2014; Coalova *et al*., 2014; Ilboudo *et al*., 2014). However, the use of 6-or 12-well plates should be considered due to their larger surface volume compared to 24-well plates. It can be hypothesised that the size of the measured response relates to the number of cells present. Although the optimal seeding density is different between cell lines, the potential effect of the seeding density on the results obtained cannot be overlooked. What is more, oxidative damage endpoints such as LPO, are usually higher in organs with oxidative potential, including the liver (Stoyanova *et al*., 2020). Lipid peroxidation in liver tissue has been well-documented (Ojha *et al*., 2011; Clasen *et al*., 2018; Nnadi *et al*., 2018) and is often used as an indicator of ecosystem health (Richardson *et al*., 2010).

5.9 Damage to the non-neuronal cholinergic system

Acetylcholinesterase is the enzyme responsible for hydrolysing the neurotransmitter, AChE, into choline and acetate (Quinn, 1987; Lionetto *et al*., 2013). Inhibition of AChE is a well-known mechanism of action of OP pesticides and is most often used as a biomarker for neurotoxicity (El-Demerdash, 2011). During the assessment of neurotoxicity of environmental pollutants, SH-SY5Y human neuroblastoma cells are usually used (Sanfeliu *et al*., 2001; Santillo & Liu, 2015) since neuronal ACh and AChE are produced within the central and peripheral nervous systems (Gu & Wang, 2021; Onder *et al*., 2022). However, variable amounts of AChE are also produced outside of the nervous system—in the NNCS (Pickett *et al*., 2017). Several organs are known to produce non-neuronal AChE: heart, kidney, ovary cells, vagina, placenta, pancreas, intestines, and liver (Berninsone *et al*., 1989; Wessler *et al*., 1998; García-Ayllón *et al*., 2012; Beckmann & Lips, 2013; Pérez-Aguilar *et al*., 2015). Moreover, Costa *et al*. (2011) state that the use of non-neuronal cells for the assessment of AChE activity *in vitro* is useful as it provides initial information regarding the effects of environmental pollutants. In this study non-neuronal cholinergic AChE activity was measured in two cell lines derived from detoxification organs: the human intestinal HuTu-80 cells and rat liver H4IIE-*luc* cells. Based on the results of this study, no quantifiable amounts of non-neuronal AChE activity were detected in the HuTu-80 cells (results not shown). A plausible explanation is the occurrence of two types of cholinesterases in mammals: acetylcholinesterase and butyrylcholinesterase (BChE) (Severi *et al*., 2022). Although AChE is present in the intestines (García-Ayllón, *et al*., 2012), ubiquitous levels of BChE have been reported along the GIT (Severi *et al*., 2022). Therefore, the amount of AChE produced by the human

intestinal cells (HuTu-80) in this study may have been too low to detect. This could be especially true since not even the basal levels of AChE produced by untreated cells were quantifiable.

On the other hand, the H4IIE-*luc* cells produced a detectable amount of AChE (**Figure 35**; **Table 19**; **Table S12**). Although BChE expression is especially high in the mouse liver (Severi *et al*., 2022), García-Ayllón *et al*. (2006) states that BChE activity in rat liver is lower than in human serum. Since the H4IIE-*luc* cells are rat liver cells, they likely produce very low levels of BChE, which allowed for the detection of AChE activity. In the present study, selected samples (M10, M12–M14) caused a statistically significant reduction in AChE activity (**Figure 35**), suggesting potential AChE inhibition.

Although neuronal AChE inhibition has been well-studied *in vitro* (Garcimartín *et al*., 2017; Fraser *et al*., 2019; Li *et al*., 2021b), a limited number of studies have reported on non-neuronal AChE inhibition and therefore comparable literature was limited. Toledo-Ibarra *et al*. (2021) found that 1 and 10 µM of the pesticide diazoxon significantly inhibited non-neuronal AChE activity *in vitro* in mononuclear spleen cells of Nile tilapia (*Oreochromis niloticus*). However, diazoxon was not present in any of the samples (**Tables S13–S14**). On the other hand, quintiophos was amongst the top ten compounds detected in M10, and M12–M14 in terms of abundance (> 40 000; **Table S13**). This insecticide elicits its mode of action via cholinesterase inhibition (Baker *et al*., 1978). However, although quintiophos was historically used in South Africa as an acaricide, resistance towards it was reported in the country for the blue tick, *Boophilus decoloratus* (Koch) (Baker *et al*., 1978). Moreover, dicamba was present in M13 and M14, but not M10 and M12 (**Table 16**). It is possible that the dicamba in the samples could have affected AChE activity. Attademo *et al*. (2021) investigated the effect of a commercial dicamba formulation (Cowboy Elite SURCOS®) on the tadpoles of two amphibian species (*Scinax nasicus* and *Elachistocleis bicolor*) by assessing several biochemical endpoints, including antioxidant enzyme responses and AChE activity. After 48 hours of exposure to sublethal dicamba concentrations, oxidative stress was induced and AChE activity was reduced in both species (Attademo *et al*., 2021). Nonetheless, it seems that any change in AChE activity (increase or decrease) could cause dysfunction of the NNCS. This is worrisome as dysfunction of the NNCS plays a role in the pathogenesis of disease (Wessler & Kirkpatrick, 2008).

5.10 Comparison between land uses and irrigation practices of sampling locations

It is possible that different land use types and irrigation practices can influence the chemical profile present at sites which are ultimately responsible for different biological effects. For example, oestrogenic endocrine disruption in fish species showed a positive association with the percentage of cultivated land and pesticide application (Blazer *et al*., 2021). In the current study there were no notable differences in the biological effects observed between MP and VH samples (**Table 19**). For MP the percentage of samples that showed an effect for endocrine disruption (55%), oxidative stress and damage (100%), and damage to the NNCS (18%) were comparable to that of the VH samples: endocrine disruption (50%), oxidative stress and damage

(100%), and NNCS damage (38%) (**Table 19**). This is an interesting observation because it was expected that the two sampling locations selected would show different responses due to their different land use and irrigation practices. The MP is mainly rainfed and is South Africa's largest maize-producing region (Grain SA, 2021). The MP sampling locations were surrounded by extensive agricultural activities (**Figure 11**), as opposed to the VH samples with considerably less (**Figure 12**). The MP is also heavily impacted by extensive mining activities that have been ongoing for more than 100 years (Vermeulen & Usher, 2009). According to Simpson *et al*. (2019), 60% of the province's surface area is subjected to mining or prospecting. This includes platinum, gold, silica, copper, iron, and coal mining. An estimated 95% of Africa's coal reserves are in South Africa and approximately 83% of this coal is mined in the MP. Consequently, the MP has a high density of coal power stations strategically located close to the coal mines—and near some of the sampling locations of this study (**Figure 11**). Moreover, the world's largest coal-to-liquid fuel plant (Secunda CTL) is also located in the MP (Simpson *et al*., 2019). Unfortunately, the water quality of water bodies in the province, including the Olifants River catchment, has been impacted by acid mine drainage and/or contaminated runoff from industrial and agricultural activities (McCarthy, 2011). Mining and industrial activities are known to release several toxic chemicals, including heavy metals (Streets *et al*., 2011) and PAHs (Liu *et al*., 2012), which have been reported to elicit biological effects (Iavicoli *et al*., 2009; Zhang *et al*., 2016; Paithankar *et al*., 2021). Although the extensive application of agrochemicals is most likely responsible for the biological effects observed for the MP samples, the influences of mining activities cannot be ruled out.

The VH, on the other hand, is less industrialised than the MP with no visible mining activities in the area (**Figure 12**). Farms in the VH mostly rely on centre pivot (maize fields) or non-pivot irrigation (pecan orchards), such as drip irrigation, due to the effectiveness of these systems (Verwey & Vermeulen, 2011). Water from a weir in the Lower Vaal River is redirected into a series of canals to irrigate crops, forming the Vaalharts Irrigation Scheme which drains into the Harts River (Van Rensburg *et al*., 2011; Verwey & Vermeulen, 2011). It is plausible that the irrigation water itself is a source of the observed biological effects for the VH samples as the Vaal River is heavily polluted (Wepener *et al*., 2011; Pheiffer *et al*., 2018; Connell *et al*., 2020; Phele *et al*., 2020; Weideman *et al*., 2020). Consequently, herbicides and other chemicals can accumulate in the water and enter soil environments following irrigation.

CHAPTER 6: CONCLUSION

6.1 General conclusion

Agrochemicals are extensively applied in South Africa. Following irrigation or heavy rainfall, many water-soluble chemicals can leach or run-off from the plants and/or soil after application. Consequently, contaminating off-site locations and potentially eliciting biological effects on non-target organisms. Since applied agrochemicals end up in the environment as complex mixtures, they can have combined effects on ecosystem health. Generally, environmental contamination is investigated by either i) instrumental chemical analysis, or ii) biological assays. However, these one-sided approaches have their disadvantages. Instrumental chemical analysis is used to determine which chemicals are present (qualitative) and in what concentration (quantitative), but it doesn't provide any information on the biological effects the chemical(s) may have on non-target organisms. Bioassays, on the other hand, are a useful tool to determine the specific effects (e.g., cytotoxicity, xenobiotic metabolism, endocrine disruption, oxidative stress and damage, inhibition of non-neuronal AChE) on non-target organisms. In some instances, the biological effects can be related back to a specific class of chemicals, for example, AhR agonism usually indicates the presence of hydrophobic and lipophilic dioxin like compounds, while endocrine disruptive effects are due to the presence of EDCs. However, the identity and concentration of the responsible chemical(s) cannot be elucidated by bioassays alone.

During the current study, the biological effects associated with the water-soluble fraction of maize field and pecan orchard soil were investigated. Polar compounds present in the soil were extracted and underwent instrument chemical analysis to identify the pesticides present. The biological effects and chemicals present were also compared between two different sampling locations: the MP, which is impacted by extensive agricultural and mining activities, and the VH with fewer anthropogenic inputs. In addition, rainfed cultivation practices are mainly followed in the MP, while farms in the VH are mostly irrigated. It is possible that different irrigation practices influence the movement of water-soluble chemicals through the soil environment due to the frequency of irrigation or rainfall events. This may affect the chemicals detected and biological effects observed in samples. Although, the MP had an overall higher pesticide load compared to the VH, there were not any considerable differences in terms of biological responses between the two sampling locations of the present study.

Although the suitability of *in vitro* methods for the assessment of biological effects of soil samples was not a specific objective of the present study, results showed that EBMs are a powerful too that can be used to assess toxicological endpoints of environmental samples (low concentrations). Although *in vitro* bioassays are more accessible for developing countries such as South Africa, compared to the use of specialised analytical instrumentation, the combined use of instrumental chemicals analysis and biological assays provides a more holistic overview of the effects of agriculture on non-target organisms in South Africa.

This study demonstrated that agricultural soils in South Africa contain quantifiable levels of water-soluble pesticides, and that the water-soluble fraction of these soils elicits biological effects *in vitro*, posing a threat to non-target organisms. Aquatic organisms are especially at risk since these polar compounds are transported to waterbodies through rainfall or irrigation. Although *in vitro* does not directly translate into *in vivo* effects, the findings of this study contribute to the general understanding of the biological effects associated with exposure to water-soluble agrochemicals in South Africa.

6.2 Study limitations

Although the results obtained in this study will undoubtedly contribute to the understanding of the water-soluble pesticides present in agricultural soils and the associated biological effects, this study did have some limitations:

- Soil samples used in this study were collected during the period of herbicide application in the maize growing season. A great addition to the outcomes of this project would be to compare results of soil sampled before and long after (end of the growing season) pesticide application, and between the wet and dry seasons.
- During sampling, no pesticide use data was collected from any farmers in the vicinity of the sampling locations. The pesticide use data could have been used to make comparisons with the results obtained from the instrumental chemical analysis, as well as investigate the environmental persistence of certain agrochemicals. It could also act as a guide for which compounds to include in targeted instrumental chemical analysis.
- Since phenol red can elicit oestrogenic effects, the use of L-15 medium without phenol red is advised for the MDA-kb2 bioassay.

6.3 Recommendations and future perspectives

Based on the results obtained in this study, conclusions arrived at, and limitations identified, several recommendations can be made for future avenue of research:

- The data obtained from the chemical screening can be used to prioritise certain chemicals for targeted analysis and create a short-list for future investigations.
- To improve the low recoveries (%) obtained for the four target pesticides during the instrumental chemical analysis, the extraction and quantification methods should be optimised for each specific compound individually.
- Even though 2,4-D, atrazine, dicamba, and imidacloprid were quantified in the soil samples, none of the observed biological effects can be directly attributed to the presence of these pesticides. It is thus recommended to evaluate the biological effects of these four pesticides *in vitro*. Treatments should include the pure compounds (i.e., active ingredients), commercial formulations, and mixtures.

- Although none of the samples contained AhR agonists, the possibility of potential AhR antagonists should be investigated.
- Since it is known that environmental mixtures may contain ligands of several different hormone receptors, it is recommended to evaluate the agonistic and antagonistic effects of other hormone receptors, such as the mineralocorticoid, progesterone, and thyroid hormone receptors.
- Pesticides can induce endocrine disruptive effects via several other mechanisms of action and not just receptor binding and/or blocking. One of these mechanisms include changes in hormone synthesis. Therefore, the effect of the water-soluble compounds present in agricultural on the steroidogenesis pathway should be investigated.
- Because of the low cellular responses obtained during the ROS, SOD, CAT, and AChE bioassays some adjustments should be made to the experimental protocol, including the use of 6-or 12-well cell culture microplates instead of 24-well plates to increase the concentration of cells.
- Experiments should be performed to determine the optimal seeding density of the HuTu-80 and H4IIE-*luc* cells and future seeding densities used for the assessment of oxidative stress responses should be adjusted accordingly.
- As only low levels of AChE were quantified in the H4IIE-*luc* cells following exposure to the soil samples, it is recommended to also investigate BChE activity in the rat liver. This can be accomplished by using a BChE selective substrate (e.g., butyrylthiocholine iodide) instead of an AChE selective substrate (i.e., acetylthiocholine iodide) during the Ellman's bioassay.
- Due to the interconnectedness of the antioxidant defence system, the effect of the samples on other oxidative stress endpoints, including GSH, GSSG, glutathione reductase, glutathione-S-transferase, and glutathione peroxidase, should also be investigated.
- Other oxidative damage endpoints must also be assessed, such as DNA damage (genotoxicity).
- Since bioassays cannot be used to determine which chemical(s) are responsible for the effects observed in the present study, the soil samples can be further processed, for example by fractionation into polar and non-polar fractions. The biological effects of the individual fractions can then be evaluated using *in vitro* bioassays in order to identify the responsible chemicals (so-called effects-directed analysis).
- As previously mentioned, *in vitro* responses cannot directly be related to *in vivo* responses since cell cultures lack the complexity of whole organisms. Therefore, the biological effects associated with agricultural soil in South Africa should be evaluated *in vivo* using test organisms that represent different trophic levels, such as green algae (primary producers), water fleas (herbivores), and fish species (higher order herbivores).

REFERENCES

Aarts, J.M.M.J.G., Denison, M.S., Cox, M.A., Schalk, M.A.C., Garrison, P.M., Tullis, K., de Haan, L.H.J. & Brouwer, A. (1995). Species-specific antagonism of Ah receptor action by 2,2′,5,5′-tetrachloro- and 2,2′,3,3′,4,4′-hexachlorobiphenyl. *European Journal of Pharmacology: Environmental Toxicology and Pharmacology Section*, 293:463–474. https://doi.org/10.1016/0926-6917(95)90067-5

Aarts, J.M.M.J.G., Denison, M.S., de Haan, L.H.J., Schalk, J.A.C., Cox, M.A. & Brouwer, A. (1993). Ah receptor-mediated luciferase expression: a tool for monitoring dioxin-like toxicity. *Organohalogen Compounds*, 13:361–364.

Abbas, A., Schneider, I., Bollmann, A., Funke, J., Oehlmann, J., Prasse, C., Schulte-Oehlmann, U., Seitz, W., Ternes, T., Weber, M., Wesely, H. & Wagner, M. (2019). What you extract is what you see: Optimising the preparation of water and wastewater samples for in vitro bioassays. *Water Research*, 152:47–60. https://doi.org/10.1016/j.watres.2018.12.049

Abdel-Halim, K.Y. & Osman, S.R. (2020). Cytotoxicity and oxidative stress responses of imidacloprid and glyphosate in human prostate epithelial WPM-Y.1 cell line. *Journal of Toxicology*, 2020: 4364650. https://doi.org/10.1155/2020/4364650

Abu Zeid, E.H., Alam, R.T.M., Ali, S.A. & Hendawi, M.Y. (2019). Dose-related impacts of imidacloprid oral intoxication on brain and liver of rock pigeon (*Columba livia domestica*), residues analysis in different organs. *Ecotoxicology and Environmental Safety*, 167:60–68. https://doi.org/10.1016/j.ecoenv.2018.09.121

Adisa, O.M., Botai, J.O., Hassen, A., Darkey, D., Adeola, A.M., Tesfamariam, E., Botai, C.M. & Adisa, A.T. (2018). Variability of satellite derived phenological parameters across maize producing areas of South Africa. *Sustainability*, 10:3033. https://doi.org/10.3390/su10093033

Agrawal, A. & Sharma, B. 2010. Pesticides induced oxidative stress in mammalian systems. *International Journal of Biological & Medical Research*, 1(3):90–104.

Aït-Aïssa, S., Laskowski, S., Laville, N., Porcher, J.-M. & Brion, F. (2010). Anti-androgenic activities of environmental pesticides in the MDA-kb2 reporter cell line. *Toxicology in Vitro*, 24:1979–1985. https://doi.org/10.1016/j.tiv.2010.08.014

Akbel, E., Arslan-Acaroz, D., Demirel, H.H., Kucukkurt, I. & Ince, S. (2018). The subchronic exposure to malathion, an organophosphate pesticide, causes lipid peroxidation, oxidative stress, and tissue damage in rats: the protective role of resveratrol. *Toxicology Research*, 7(3):503–512. https://doi.org/10.1039/C8TX00030A

Akoglu, H. (2018). User's guide to correlation coefficients. *Turkish Journal of Emergency Medicine*, 18:91–93. https://doi.org/10.1016/j.tjem.2018.08.001

Allen, D.R. & McWhinney, B.C. (2019). Quadrupole time-of-flight mass spectrometry: a paradigm shift in toxicology screening applications. *Clinical Biochemist Reviews*, 40(3):135–146. https://doi.org/10.33176/AACB-19-00023

Almroth, B.C., Sturve, J., Berglund, Å. & Förlin, L. (2005). Oxidative damage in eelpout (*Zoarces viviparus*), measured as protein carbonyls and TBARS, as biomarkers. *Aquatic Toxicology*, 73:171–180. https://doi.org/10.1016/j.aquatox.2005.03.007

Alvarez, D.A., Shappell, N.W., Billey, L.O., Bermudez, D.S., Wilson, V.S., Kolpin, D.W., Perkins, S.D., Evans, N., Foreman, W.T., Gray, J.L., Shipitalo, M.J. & Meyer, M.T. (2013). Bioassay of estrogenicity and chemical

analyses of estrogens in streams across the United States associated with livestock operations. *Water Research*, 47:3347–3363. https://doi.org/10.1016/j.watres.2013.03.028

Alvarez-Muñoz, D., Indiveri, P., Rostkowski, P., Horwood, J., Greer, E., Minier, C., Pope, N., Langston, W.J. & Hill, E.M. (2015). Widespread contamination of coastal sediments in the Transmanche Channel with anti-androgenic compounds. *Marine Pollution Bulletin*, 95:590–597. https://doi.org/10.1016/j.marpolbul.2014.11.014

Andersen, H.R., Vinggaard, A.M., Høj Rasmussen, T., Gjermandsen, I.M. & Bonefeld-Jørgensen, C.E. (2002). Effects of currently used pesticides in assays for estrogenicity, androgenicity, and aromatase activity *in vitro*. *Toxicology and Applied Pharmacology*, 179:1–12. https://doi.org/10.1006/taap.2001.9347

Andersen, L., Goto-Kazeto, R., Trant, J. M., Nash, J. P., Korsgaard, B. & Bjerregaard, P. (2006). Short-term exposure to low concentrations of the synthetic androgen methyltestosterone affects vitellogenin and steroid levels in adult male zebrafish (*Danio rerio*). *Aquatic Toxicology*, 76:343–352. https://doi.org/10.1016/j.aquatox.2005.10.008

Aneck-Hahn, N.H, Bornman, M.S. & de Jager, C. (2008). Preliminary assessment of oestrogenic activity in water sources in Rietvlei Nature Reserve, Gauteng, South Africa. *African Journal of Aquatic Science*, 33:249–254. https://doi.org/10.2989/AJAS.2008.33.3.7.619

Aneck-Hahn, N.H., Bornman, M.S., & de Jager, C. (2009). Oestrogenic activity in drinking waters from a rural area in the Waterberg District, Limpopo Province, South Africa. *Water SA*, 35(3):245–252. https://doi.org/10.4314/wsa.v35i3.76760

Aneck-Hahn, N.H., de Jager, C., Bornman, M.S. & du Toit, D. (2005). Oestrogenic activity using a recombinant yeast screen assay (RCBA) in South African laboratory water sources. *Water SA*, 31(2):253–256. https://doi.org/10.4314/wsa.v31i2.5192

Aneck-Hahn, N.H., van Zijl, M.C., Swart, P., Truebody, B., Genthe, B., Charmier, J. & Jager, C. de. (2018). Estrogenic activity, selected plasticizers and potential health risks associated with bottled water in South Africa. *Journal of Water and Health*, 16(2):253–262. https://doi.org/10.2166/wh.2018.043

Ansari, S. & Ansari, B.A. (2014). Temporal variations of CAT, GSH, and LPO in gills and livers of zebrafish, *Danio rerio*, exposed to dimethoate. *Archives of Polish Fisheries*, 22:101–109. https://doi.org/10.2478/aopf-2014-0009

Archer, E. & van Wyk, J.H. (2015). The potential anti-androgenic effect of agricultural pesticides used in the Western Cape: *In vitro* investigation of mixture effects. *Water SA*, 41(1):129–137. https://doi.org/10.4314/wsa.v41i1.16

Archer, E., Wolfaardt, G.M. & Van Wyk, J.H. 2017. Pharmaceutical and personal care products (PPCPs) as endocrine disrupting contaminants (EDCs) in South African surface waters. *Water SA*, 43(4):684. https://doi.org/10.4314/wsa.v43i4.16

Archibong, A.E., Rideout, M.L., Harris, K J. & Ramesh, A. (2018). Oxidative stress in reproductive toxicology. *Current Opinion in Toxicology*, 7:95–101. https://doi.org/10.1016/j.cotox.2017.10.004

Arukwe, A., Myburgh, J., Langberg, H.A., Adeogun, A.O., Braa, I.G., Moeder, M., Schlenk, D., Crago, J.P., Regoli, F. & Botha, C. (2016). Developmental alterations and endocrine-disruptive responses in farmed Nile crocodiles (*Crocodylus niloticus*) exposed to contaminants from the Crocodile River, South Africa. *Aquatic Toxicology*, 173:83–93. https://doi.org/10.1016/j.aquatox.2015.12.027

Astiz, M., de Alaniz, M.J.T., & Marra, C.A. (2009). The impact of simultaneous intoxication with agrochemicals on the antioxidant defense system in rat. *Pesticide Biochemistry and Physiology*, 94(2–3):93–99. https://doi.org/10.1016/j.pestbp.2009.03.005

Atalay, A. & Hwang, K.-J. (1999). Extractability of 2,4-D, dicamba and MCPP from soil. *Water, Ai, and Soil Pollution*, 114:155–170.

Attademo, A.M., Lajmanovich, R.C., Peltzer, P.M., Paula, A., Boccioni, C., Martinuzzi, C., Simonielo, F. & Repetti, M.R. (2021). Effects of the emulsifiable herbicide Dicamba on amphibian tadpoles: an underestimated toxicity risk? *Environmental Science and Pollution Research*, *28*, 31962–31974. https://doi.org/10.1007/s11356-021-13000-x/Published

Avilla, M.N., Malecki, K.M.C., Hahn, M.E., Wilson, R.H. & Bradfield, C.A. (2020). The Ah receptor: adaptive metabolism, ligand diversity, and the xenokine model. *Chemical Research in Toxicology*, 33(4):860–879. https://doi.org/10.1021/acs.chemrestox.9b00476.

Baba, T., Mimura, J., Nakamura, N., Harada, N., Yamamoto, M., Morohashi, K. & Fujii-Kuriyama, Y. (2005). Intrinsic function of the aryl hydrocarbon (dioxin) receptor as a key factor in female reproduction. *Molecular and Cellular Biology*, 25(22):10040–10051. https://doi.org/10.1128/MCB.25.22.10040

Baines, D., Wilton, E., Pawluk, A., de Gorter, M. & Chomistek, N. (2017). Neonicotinoids act like endocrine disrupting chemicals in newly-emerged bees and winter bees. *Scientific Reports*, 7:10979. https://doi.org/10.1038/s41598-017-10489-6

Baker, J.A.F., Miles, J.O., Robertson, W.D., Stanford, G.D. & Taylor, R.J. (1978). The current status of resistance to organophosphorus ixodicides by the blue tick, *Boophilus decoloratus* (Koch) in the republic of South Africa and Transkei. *Journal of the South African Veterinary Association*, 49(4):327–333.

Bali, Y.A., Kaikai, N., Ba-M'hamed, S. & Bennis, M. (2019). Learning and memory impairments associated to acetylcholinesterase inhibition and oxidative stress following glyphosate based-herbicide exposure in mice. *Toxicology*, 415:18–25. https://doi.org/10.1016/j.tox.2019.01.010

Barouki, R., Coumoul, X. & Fernandez-Salguero, P.M. (2007). The aryl hydrocarbon receptor, more than a xenobiotic-interacting protein. *FEBS Letters*, 581:3608–3615. https://doi.org/10.1016/j.febslet.2007.03.046

Barnhoorn, I.E.J., Bornman, M.S., Pieterse, G.M., & van Vuren, J.H.J. (2004). Histological evidence of intersex in feral sharptooth catfish (*Clarias gariepinus*) from an estrogen-polluted water source in Gauteng, South Africa. *Environmental Toxicology*, 19(6):603–608. https://doi.org/10.1002/tox.20068

Baruah, P. & Chaurasia, N. 2020. Ecotoxicological effects of alpha-cypermethrin on freshwater alga *Chlorella* sp.: Growth inhibition and oxidative stress studies. *Environmental Toxicology and Pharmacology*, 76:103347. https://doi.org/10.1016/j.etap.2020.103347

Basopo, N. & Muzvidziwa, A. (2020). Assessment of the effects of atrazine, dichlorodiphenyltrichloroethane, and dimethoate on freshwater fish (*Oreochromis mossambicus*): a case study of the A2 farmlands in Chiredzi, in the southeastern part of Zimbabwe. *Environmental Science and Pollution Research*, 27(1):579–586. https://doi.org/10.1007/s11356-019-06569-x

Basopo, N., Langton, T.M., Dumisani, M. & Yogeshkumar, S. N. (2014). Pollutant mixtures as stressors of selected enzyme activities of the aquatic snail *Helisoma duryi*. *Journal of Environmental Chemistry and Ecotoxicology*, 6(4):27–37. https://doi.org/10.5897/JECE2013.0319

Bebe, F.N. & Panemangalore, M. (2003). Exposure to low doses of endosulfan and chlorpyrifos modifies endogenous antioxidants in tissues of rats. *Journal of Environmental Science and Health, Part B*, 38(3):349–363. https://doi.org/10.1081/PFC-120019901

Beckmann, J. & Lips, K. S. (2013). The non-neuronal cholinergic system in health and disease. *Pharmacology*, 92:286–302. https://doi.org/10.1159/000355835

Belaid, A., Methneni, N., Nasri, E., Bchir, S., Anthonissen, R., Verschaeve, L., Le Tilly, V., Lo Turco, V., Di Bella, G., Mansour, H.B. & Nezar, H.K. (2021). Endocrine disruption, cytotoxicity and genotoxicity of an organophosphorus insecticide. *Sustainability*, 13:11512. https://doi.org/10.3390/su132011512

Benzie, I. F. F. (2000). Evolution of antioxidant defence mechanisms. *European Journal of Nutrition*, 39:53–61. https://doi.org/10.1007/s003940070030

Berninsone, P., Katz, E., Napp, M. & Azcurra, J. (1989). Acetylcholinesterase and nonspecific cholinesterase activities in rat liver: subcellular localization, molecular forms, and some extraction properties. *Biochemistry and Cell Biology*, 67:817–822.

Berridge, M.V., Herst, P.M. & Tan, A.S. (2005). Tetrazolium dyes as tools in cell biology: New insights into their cellular reduction. *Biotechnology Annual Review*, 11:127–152. https://doi.org/10.1016/S1387-2656(05)11004-7

Berthois, Y., Katzenellenbogent, J.A. & Katzenellenbogen, B.S. (1986). Phenol red in tissue culture media is a weak estrogen: Implications concerning the study of estrogen-responsive cells in culture (cell proliferation/human breast cancer/antiestrogens/hormone responsiveness/estrogen receptor). *Cell Biology*, 83:2496–2500.

Bigas, J., Sevilla, L.M., Carceller, E., Boix, J. & Pérez, P. (2018). Epidermal glucocorticoid and mineralocorticoid receptors act cooperatively to regulate epidermal development and counteract skin inflammation article. *Cell Death and Disease*, 9:588. https://doi.org/10.1038/s41419-018-0673-z

Bindoli, A. (1988). Lipid peroxidation in mitochondria. *Free Radical Biology and Medicine*, 5:247–261. https://doi.org/10.1177/1461444810365020

Bish, M.D., Farrell, S.T., Lerch, R.N. & Bradley, K.W. (2019). Dicamba losses to air after applications to soybean under stable and nonstable atmospheric conditions. *Journal of Environmental Quality*, 48(6):1675–1682. https://doi.org/10.2134/jeq2019.05.0197

Björnström, L. & Sjöberg, M. (2005). Mechanisms of estrogen receptor signaling: Convergence of genomic and nongenomic actions on target genes. *Molecular Endocrinology*, 19(4):833–842. https://doi.org/10.1210/me.2004-0486

Blahová, J., Plhalová, L., Hostovský, M., Divišová, L., Dobšíková, R., Mikulíková, I., Štěpánová, S. & Svobodová, Z. (2013). Oxidative stress responses in zebrafish *Danio rerio* after subchronic exposure to atrazine. *Food and Chemical Toxicology*, 61:82–85. https://doi.org/10.1016/j.fct.2013.02.041

Blake, L.S., Martinović, D., Gray, L.E., Wilson, V.S., Regal, R.R., Villeneuve, D.L. & Ankley, G.T. (2010). Characterization of the androgen-sensitive MDA-kb2 cell line for assessing complex environmental mixtures. *Environmental Toxicology and Chemistry*, 29(6):1367–1376. https://doi.org/10.1002/etc.166

Blazer, V.S., Gordon, S., Jones, D.K., Iwanowicz, L.R., Walsh, H.L., Sperry, A.J. & Smalling, K.L. (2021). Retrospective analysis of estrogenic endocrine disruption and land-use influences in the Chesapeake Bay watershed. *Chemosphere*, 266:129009. https://doi.org/10.1016/j.chemosphere.2020.129009

Bonmatin, J.M., Moineau, I., Charvet, R., Fleche, C., Colin, M.E. & Bengsch, E.R. (2003). A LC/APCI-MS/MS method for analysis of imidacloprid in soils, in plants, and in pollens. *Analytical Chemistry*, 75:2027–2033. https://doi.org/10.1021/ac020600b

Bornman, M.S., Barnhoorn, I.E.J., de Jager, C. & Veeramachaneni, D.N.R. (2010). Testicular microlithiasis and neoplastic lesions in wild eland (*Tragelaphus oryx*): Possible effects of exposure to environmental pollutants? *Environmental Research*, 110(4):327–333. https://doi.org/10.1016/j.envres.2010.02.003

Botha, C.J., du Plessis, E.C., Coetser, H. & Rosemann, M. (2018). Analytical confirmation of imidacloprid poisoning in granivorous Cape spurfowl (*Pternistis capensis*). *Journal of the South African Veterinary Association*, 89:a1637. https://doi.org/10.4102/jsava.v89i0.1637

Boussabbeh, M., ben Salem, I., Hamdi, M., ben Fradj, S., Abid-Essefi, S. & Bacha, H. (2016). Diazinon, an organophosphate pesticide, induces oxidative stress and genotoxicity in cells deriving from large intestine. *Environmental Science and Pollution Research*, 23:2882–2889. https://doi.org/10.1007/s11356-015-5519-y

Boyer, J.N. & Groffman, P.M. (1996). Bioavailability of water extractable organic carbon fractions in forest and agricultural soil profiles. *Soil Biology and Biochemistry*, 28(6):783–790.

Brack, W., Ait-Aissa, S., Backhaus, T., Dulio, V., Escher, B.I., Faust, M., Hilscherova, K., Hollender, J., Hollert, H., Müller, C., Munthe, J., Leo Posthuma, Thomas-Benjamin Seiler, J.S., Teodorovic, I., Tindall, A.J., de Aragão Umbuzeiro, Gisela Zhang, X. & Altenburger, R. (2019). Effect-based methods are key. The European Collaborative Project SOLUTIONS recommends integrating effect-based methods for diagnosis and monitoring of water quality. *Environmental Sciences Europe*, 31:10. https://doi.org/10.1186/s12302-019-0192-2

Brack, W., Escher, B.I., Müller, E., Schmitt-Jansen, M., Schulze, T., Slobodnik, J. & Hollert, H. (2018). Towards a holistic and solution-oriented monitoring of chemical status of European water bodies: how to support the EU strategy for a non-toxic environment? *Environmental Sciences Europe*, 30:33. https://doi.org/10.1186/s12302-018-0161-1

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1–2):248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Burella, P.M., Odetti, L.M., Simoniello, M.F. & Poletta, G.L. (2018). Oxidative damage and antioxidant defense in *Caiman latirostris* (Broad-snouted caiman) exposed *in ovo* to pesticide formulations. *Ecotoxicology and Environmental Safety*, 161:437–443. https://doi.org/10.1016/j.ecoenv.2018.06.006

Capkin, E. & Altinok, I. (2013). Effects of chronic carbosulfan exposure on liver antioxidant enzyme activities in rainbow trout. *Environmental Toxicology and Pharmacology*, 36:80–87. https://doi.org/10.1016/j.etap.2013.03.022

Capkin, E., Boran, H. & Altinok, I. (2014). Response of acetylcholinesterase (AChE) in the erythrocyte and liver of rainbow trout exposed to carbosulfan. *Turkish Journal of Fisheries and Aquatic Sciences*, 14:643–650. https://doi.org/10.4194/1303-2712-v14_3_06

Casey, F.X.M., Hakk, H., Šimůnek, J. & Larsen, G.L. (2004). Fate and transport of testosterone in agricultural soils. *Environmental Science and Technology*, 38(3):790–798. https://doi.org/10.1021/es034822i

Celma, A., Mandava, G., Oskarsson, A., Sancho, J.V., Bijlsma, L. & Lundqvist, J. (2021). *In vitro* bioanalytical assessment of toxicity of wetland samples from Spanish Mediterranean coastline. *Environmental Sciences Europe*, 33:70. https://doi.org/10.1186/s12302-021-00510-1

Cha, J., Hong, S., Gwak, J., Kim, M., Lee, J., Kim, T., Han, G.M., Hong, S.H., Hur, J., Giesy, J.P. & Khim, J.S. (2022). Identification of novel polar aryl hydrocarbon receptor agonists accumulated in liver of black-tailed gulls in Korea using advanced effect-directed analysis. *Journal of Hazardous Materials*, 429. https://doi.org/10.1016/j.jhazmat.2022.128305.

Chance, B. (1948). The enzyme-substrate compounds of catalase and peroxides. *Nature*, 161:914–917. https://doi.org/10.1038/161914a0

Chang, H., Hu, J. & Shao, B. (2007). Occurrence of natural and synthetic glucocorticoids in sewage treatment plants and receiving river waters. *Environmental Science and Technology*, 41:3462–3468. https://doi.org/10.1021/es062746o

Chang, X., Wang, X., Feng, J., Su, X., Liang, J., Li, H. & Zhang, J. (2020). Impact of chronic exposure to trichlorfon on intestinal barrier, oxidative stress, inflammatory response and intestinal microbiome in common carp (*Cyprinus carpio* L.). *Environmental Pollution*, 259:113846. https://doi.org/10.1016/j.envpol.2019.113846

Chaufan, G., Coalova, I. & del Carmen Ríos de Molina, M. (2014). Glyphosate commercial formulation causes cytotoxicity, oxidative effects, and apoptosis on human cells: differences with its active ingredient. *International Journal of Toxicology*, 33(1):29–38. https://doi.org/10.1177/1091581813517906

Chen, D., Zhang, Z., Yao, H., Liang, Y., Xing, H. & Xu, S. (2015). Effects of atrazine and chlorpyrifos on oxidative stress-induced autophagy in the immune organs of common carp (*Cyprinus carpio* L.). *Fish & Shellfish Immunology*, 44:12–20. https://doi.org/10.1016/j.fsi.2015.01.014

Chen, J.R., Lazarenko, O.P., Haley, R.L., Blackburn, M.L., Badger, T.M. & Ronis, M. J. (2009). Ethanol impairs estrogen receptor signaling resulting in accelerated activation of senescence pathways, whereas estradiol attenuates the effects of ethanol in osteoblasts. *Journal of Bone and Mineral Research*, 24(2):221–230. https://doi.org/10.1359/jbmr.081011

Chikoore, H. & Jury, M.R. (2021). South African drought, deconstructed. *Weather and Climate Extremes*, 33:100334. https://doi.org/10.1016/j.wace.2021.100334

Christiaens, V., Berckmans, P., Haelens, A., Witters, H. & Claessens, F. (2005). Comparison of different androgen bioassays in the screening for environmental (anti)androgenic activity. *Environmental Toxicology and Chemistry*, 24(10):2646–2656. https://doi.org/10.1897/05-126R.1

Clasen, B., Loro, V.L., Murussi, C.R., Tiecher, T.L., Moraes, B. & Zanella, R. (2018). Bioaccumulation and oxidative stress caused by pesticides in *Cyprinus carpio* reared in a rice-fish system. *Science of The Total Environment*, 626:737–743. https://doi.org/10.1016/j.scitotenv.2018.01.154

Coalova, I., Ríos de Molina, M. del C. & Chaufan, G. (2014). Influence of the spray adjuvant on the toxicity effects of a glyphosate formulation. *Toxicology in Vitro*, 28(7):1306–1311. https://doi.org/10.1016/j.tiv.2014.06.014

Cohen, G., Dembiec, D. & Marcus, J. (1970). Measurement of catalase activity in tissue extracts. *Analytical Biochemistry*, 34:30–38. https://doi.org/10.1016/0003-2697(70)90083-7

Coleman, O. I., & Haller, D. (2018). Bacterial signaling at the intestinal epithelial interface in inflammation and cancer. *Frontiers in Immunology*, 8:1927. https://doi.org/10.3389/fimmu.2017.01927

Conley, J.M., Evans, N., Cardon, M.C., Rosenblum, L., Iwanowicz, L.R., Hartig, P.C., Schenck, K.M., Bradley, P.M. & Wilson, V.S. (2017). Occurrence and *in vitro* bioactivity of estrogen, androgen, and glucocorticoid compounds in a nationwide screen of United States stream waters. *Environmental Science and Technology*, *51*, 4781–4791. https://doi.org/10.1021/acs.est.6b06515

Connell, L., Jansen van Rensburg, G., Avenant-Oldewage, A. & Greenfield, R. (2020). Biomarker responses in African sharptooth catfish, *Clarias gariepinus* (Burchell, 1822), as indicators of potential metal and organic pollution along the Vaal River system, South Africa. *African Journal of Aquatic Science*, 45(3):317–328. https://doi.org/10.2989/16085914.2020.1735296

Connon, R.E., Geist, J. & Werner, I. (2012). Effect-based tools for monitoring and predicting the ecotoxicological effects of chemicals in the aquatic environment. *Sensors*, 12:12741–12771. https://doi.org/10.3390/s120912741

Connor, K., Howell, J., Chen, I., Liu, H., Berhane, K., Sciarretta, C., Safe, S. & Zacharewski, T. (1996). Failure of chloro-S-triazine-derived compounds to induce estrogen receptor-mediated responses *in vivo* and *in vitro*. *Fundamental and Applied Toxicology*, 30:93–101. https://academic.oup.com/toxsci/article/30/1/93/1651188

Contardo-Jara, V. & Gessner, M.O. (2020). Uptake and physiological effects of the neonicotinoid imidacloprid and its commercial formulation Confidor® in a widespread freshwater oligochaete. *Environmental Pollution*, 264:114793. https://doi.org/10.1016/j.envpol.2020.114793

Conradie, E.H., Van Zyl, P.G., Pienaar, J.J., Beukes, J.P., Galy-Lacaux, C., Venter, A.D. & Mkhatshwa, G. V. (2016). The chemical composition and fluxes of atmospheric wet deposition at four sites in South Africa. *Atmospheric Environment*., 146:113–131. https://doi.org/10.1016/j.atmosenv.2016.07.033

Cortés-Iza, S.C. & Rodríguez, A.I. (2018). Oxidative stress and pesticide disease: a challenge for toxicology. *Revista de la Facultad de Medicina*, 66(2):261–267. https://doi.org/10.15446/revfacmed.v66n2.60783

Costa, L.G., Giordano, G. & Guizzetti, M. (2011). *In vitro* approaches to developmental neurotoxicity. (*In* Gupta, R. C., *ed*. Reproductive and developmental toxicology. Amsterdam: Elsevier. p. 159–166). https://doi.org/10.1016/B978-0-12-382032-7.10013-X

Cox, C. & Surgan, M. (2006). Unidentified inert ingredients in pesticides: implications for human and environmental health. *Environmental Health Perspectives*, 114(12): 1803–1806. https://doi.org/10.1289/ehp.9374

Curchod, L., Oltramare, C., Junghans, M., Stamm, C., Dalvie, M.A., Röösli, M. & Fuhrimann, S. (2020). Temporal variation of pesticide mixtures in rivers of three agricultural watersheds during a major drought in the Western Cape, South Africa. *Water Research X*, 6:100039. https://doi.org/10.1016/j.wroa.2019.100039

Cycoń, M., Żmijowska, A. & Piotrowska-Seget, Z. (2011). Biodegradation kinetics of 2,4-D by bacterial strains isolated from soil. *Central European Journal of Biology*, 6(2):188–198. https://doi.org/10.2478/s11535-011-0005-0

Dabrowski, J.M. (2015a). Development of pesticide use maps for South Africa. *South African Journal of Science*, 111(1–2):1–7. https://doi.org/10.17159/sajs.2015/20140091

Dabrowski, J.M. (2015b). Investigation of the contamination of water resources by agricultural chemicals and the impact on environmental health. Volume 1: Risk assessment of agricultural chemicals to human and animal health. Water Research Commission (WRC) Report No. 1956/1/15. WRC, Pretoria, South Africa.

Dabrowski, J.M. (2015c). Investigation of the contamination of water resources by agricultural chemicals and the impact on environmental health. Volume 2: Prioritizing human health effects and mapping sources of agricultural pesticides used in South Africa. Water Research Commission (WRC) Report No. TT 642/15. WRC, Pretoria, South Africa.

Dabrowski, J.M., Shadung, J.M. & Wepener, V. (2014). Prioritizing agricultural pesticides used in South Africa based on their environmental mobility and potential human health effects. *Environment International*, 62:31–40. https://doi.org/10.1016/j.envint.2013.10.001

Dang, Z.C. & Lowik, C.W.G.M. (2005). Removal of serum factors by charcoal treatment promotes adipogenesis via a MAPK-dependent pathway. *Molecular and Cellular Biochemistry*, 268:159–167. https://doi.org/10.1007/s11010-005-3857-7

Dankyi, E., Gordon, C., Carboo, D. & Fomsgaard, I. S. (2014). Quantification of neonicotinoid insecticide residues in soils from cocoa plantations using a QuEChERS extraction procedure and LC-MS/MS. *Science of the Total Environment*, 499(1):276–283. https://doi.org/10.1016/j.scitotenv.2014.08.051

Dar, S.A., Yousuf, A.R., Balkhi, M.-H., Ganai, F.A. & Bhat, F.A. (2015). Assessment of endosulfan induced genotoxicity and mutagenicity manifested by oxidative stress pathways in freshwater cyprinid fish crucian carp (*Carassius carassius* L.). *Chemosphere*, 120:273–283. https://doi.org/10.1016/j.chemosphere.2014.07.031

Darbre, P.D. (2020). Chemical components of plastics as endocrine disruptors: Overview and commentary. *Birth Defects Research*, 112:1300–1307. https://doi.org/10.1002/bdr2.1778

Da Silva, D.R.O., Cuchi, M.L., da Silva, Á.A.A., Novello, B.D. & Basso, C.J. (2020). Simulated rainfall following the preplant application of 2,4-d and dicamba in soybean. *Pesquisa Agropecuaria Tropical*, 50:e62780. https://doi.org/10.1590/1983-40632020V5062780

Da Silva, J.J., da Silva, B.F., Stradiotto, N.R., Petrović, M., Gros, M. & Gago-Ferrero, P. (2021). Identification of organic contaminants in vinasse and in soil and groundwater from fertigated sugarcane crop areas using target and suspect screening strategies. *Science of The Total Environment*, 761:143237. https://doi.org/10.1016/j.scitotenv.2020.143237

Davey, R.A. & Grossmann, M. (2016). Androgen receptor structure, function and biology: From bench to bedside. *Clinical Biochemist Reviews*, *37*(1), 3–15.

De Araújo-Ramos, A.T., Passoni, M.T., Romano, M.A., Romano, R.M. & Martino-Andrade, A.J. (2021). Controversies on endocrine and reproductive effects of glyphosate and glyphosate-based herbicides: A mini-review. *Frontiers in Endocrinology*, 12:627210. https://doi.org/10.3389/fendo.2021.627210

De Falco, M., Forte, M. & Laforgia, V. (2015). Estrogenic and anti-androgenic endocrine disrupting chemicals and their impact on the male reproductive system. *Frontiers in Environmental Science*, 3:3. https://doi.org/10.3389/fenvs.2015.00003

Debes, J.D. & Tindall, D.J. (2002). The role of androgens and the androgen receptor in prostate cancer. *Cancer Letters*, 187:1–7. https://doi.org/10.1016/S0304-3835(02)00413-5

Degrendele, C., Klánová, J., Prokeš, R., Příbylová, P., Šenk, P., Šudoma, M., Röösli, M., Dalvie, M.A. & Fuhrimann, S. (2022). Current use pesticides in soil and air from two agricultural sites in South Africa: Implications for environmental fate and human exposure. *Science of the Total Environment*, 807:150455. https://doi.org/10.1016/j.scitotenv.2021.150455

Del Maestro, R. & McDonald, W. (1987). Distribution of superoxide dismutase, glutathione peroxidase and catalase in developing rat brain. *Mechanisms of Ageing and Development*, 41:29–38. https://doi.org/10.1016/0047-6374(87)90051-0

Denison, M.S., Zhao, B., Baston, D.S., Clark, G.C., Murata, H. & Han, D. (2004). Recombinant cell bioassay systems for the detection and relative quantitation of halogenated dioxins and related chemicals. *Talanta*, 63(5):1123–1133. https://doi.org/10.1016/j.talanta.2004.05.032

Department of Water Affairs and Forestry (DWAF). (1996). South African Water Quality Guidelines. Volume 7: Aquatic Ecosystems.

Diken, M.E., Dogan, S., Dogan, M. & Turhan, Y. (2017). *In vitro* effects of some pesticides on glutathione-s transferase activity. *Fresenius Environmental Bulletin*, 25(12A):8023–8029.

Doan, T. Q., Berntsen, H. F., Verhaegen, S., Ropstad, E., Connolly, L., Igout, A., Muller, M. & Scippo, M. L. (2019). A mixture of persistent organic pollutants relevant for human exposure inhibits the transactivation activity of the aryl hydrocarbon receptor *in vitro*. *Environmental Pollution*, 254:113098. https://doi.org/10.1016/j.envpol.2019.113098

Donkin, S.G. & Williams, P. L. (2000). Neurotoxicity: Toxic responses of the nervous system. (*In* William, P.L., James, R.C. & Roberts, S.M., *eds*. Principles of toxicology: Environmental and industrial applications. New York: Wiley. p. 145–155).

Du Preez, L.H., Jansen van Rensburg, P.J., Jooste, A.M., Carr, J.A., Giesy, J.P., Gross, T.S., Kendall, R.J., Smith, E.E., van der Kraak, G. & Solomon, K.R. (2005). Seasonal exposures to triazine and other pesticides in surface waters in the western Highveld corn-production region in South Africa. *Environmental Pollution*, 135:131–141. https://doi.org/10.1016/j.envpol.2004.09.019

Durães, N., Novo, L.A.B., Candeias, C. & da Silva, E.F. (2018). Distribution, transport and fate of pollutants. (*In* Duarte, A.C., Cachada, A. & Rocha-Santos, T., *eds*. Soil pollution: from monitoring to remediation. Massachusetts: Academic Press. p. 29–57). https://doi.org/10.1016/B978-0-12-849873-6.00002-9

El-Bini Dhouib, I., Annabi, A., Jrad, A., El-Golli, N., Gharbi, N., Lasram, M.M. & El-Fazaa, S. (2015). Carbosulfan-induced oxidative damage following subchronic exposure and the protective effects of N-acetylcysteine in rats. *General Physiology and Biophysics*, 34:249–261. https://doi.org/10.4149/gpb_2015005

El-Demerdash, F.M. (2011). Lipid peroxidation, oxidative stress and acetylcholinesterase in rat brain exposed to organophosphate and pyrethroid insecticides. *Food and Chemical Toxicology*, 49:1346–1352. https://doi.org/10.1016/j.fct.2011.03.018

Eldridge, J.C., Stevens, J.T. & Breckenridge, C.B. (2008). Atrazine interaction with estrogen expression systems. (*In* Whitacre, D. *ed*., Reviews of environmental contamination and toxicology. Berlin: Springer. p. 147–160). Shttps://doi.org/10.1007/978-0-387-78444-1_6

El-Gendy, K.S., Aly, N.M., Mahmoud, F.H., Kenawy, A. & El-Sebae, A.K.H. (2010). The role of vitamin C as antioxidant in protection of oxidative stress induced by imidacloprid. *Food and Chemical Toxicology*, 48:215–221. https://doi.org/10.1016/j.fct.2009.10.003

Ellis, S.M. & Steyn, H.S. (2003). Practical significance (effect sizes) versus or in combination with statistical significance (p-values). *Management Dynamics*, *12*(4):51–53. https://www.researchgate.net/publication/293182482

Ellman, G.L., Courtney, K.D., Andres, V. & Featherstone, R.M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, 7(2):88–95. https://doi.org/10.1016/0006-2952(61)90145-9

Ermler, S., Scholze, M. & Kortenkamp, A. (2010). The sensitivity of the MDA-kb2 cell *in vitro* assay in detecting anti-androgenic chemicals - Identification of sources of variability and estimation of statistical power. *Toxicology in Vitro*, 24:1845–1853. https://doi.org/10.1016/j.tiv.2010.05.007

Escher, B.I., Allinson, M., Altenburger, R., Bain, P.A., Balaguer, P., Busch, W., Crago, J., Denslow, N.D., Dopp, E., Hilscherova, K., Humpage, A.R., Kumar, A., Grimaldi, M., Jayasinghe, B.S., Jarosova, B., Jia, A., Makarov, S., Maruya, K.A., Medvedev, A., Mehinto, A.C., Mendez, J.E., Poulsen, A., Prochazka, E., Richard, J., Schi,

A., Schlenk, D., Scholz, S., Shiraishi, F., Snyder, S., Su, G., Tang, J.Y.M., Burg, B. Van Der, Linden, S.C. Van Der, Werner, I., Westerheide, S.D., Wong, C.K.C., Yang, M., Yeung, B.H.Y., Zhang, X., & Leusch, F.D.L. (2014). Benchmarking Organic micropollutants in wastewater, recycled water and drinking water with *in vitro* bioassays. *Environmental Science and Technology*, 48:1940–1956.

Escher, B.I, Neale, P.A. & Leusch, F.D. (2021). Bioanalytical tools in water quality assessment. London: IWA Publishing.

Escher, B.I., Stapleton, H.M. & Schymanski, E.L. (2020). Tracking complex mixtures of chemicals in our changing environment. *Science*, 367(6476):388–392. https://doi.org/10.1126/science.aay6636

Eze, C.T., Otitoloju, A.A., Eze, O.O., Ugochukwu, T.E., Onodugo, C., Ali, A.M., Lyche, J.L., Karlsen, O.A. & Goksøyr, A. (2023). West African e-waste-soil assessed with a battery of cell-based bioassays. *Science of The Total Environment*, 856:159068. https://doi.org/10.1016/j.scitotenv.2022.159068

Farombi, E., Ajimoko, Y. & Adelowo, O. (2008). Effect of butachlor on antioxidant enzyme status and lipid peroxidation in fresh water African catfish, (*Clarias gariepinus*). *International Journal of Environmental Research and Public Health*, 5(5):423–427. https://doi.org/10.3390/ijerph5050423

Farounbi, A.I. & Ngqwala, N.P. (2020). Occurrence of selected endocrine disrupting compounds in the Eastern Cape province of South Africa. *Environmental Science and Pollution Research*, 27(14):17268–17279. https://doi.org/10.1007/s11356-020-08082-y

Feng, Q. & He, B. (2019). Androgen receptor signaling in the development of castration-resistant prostate cancer. *Frontiers in Oncology*, 9:858. https://doi.org/10.3389/fonc.2019.00858

Ferreira, D., Costa da Motta, A., Kreutz, L.C., Toni, C., Loro, V.L. & Barcellos, L.J.G. (2010). Assessment of oxidative stress in *Rhamdia quelen* exposed to agrichemicals. *Chemosphere*, 79(9):914–921. https://doi.org/10.1016/j.chemosphere.2010.03.024

Ferrer, I. & Thurman, E.M. (2003). Liquid chromatography/time-of-flight/mass spectrometry (LC/TOF/MS) for the analysis of emerging contaminants. *Trends in Analytical Chemistry*, 22(10):750–756. https://doi.org/10.1021/bk-2003-0850

Fischer, F.C., Henneberger, L., Schlichting, R. & Escher, B.I. (2019). How to improve the dosing of chemicals in high-throughput *in vitro* mammalian cell assays. *Chemical Research in Toxicology*, *32*(8):1462–1468. https://doi.org/10.1021/acs.chemrestox.9b00167

Food and Agriculture Organization of the United Nations Statistical Database (FAOSTAT). (2020a). Crops and livestock products. https://www.fao.org/faostat/en/#data/QCL/visualize Date of Access: 25 July 2022.

Food and Agriculture Organization of the United Nations Statistical Database (FAOSTAT). (2020b). Pesticides Use. https://www.fao.org/faostat/en/#data/RP Date of access: 25 July 2022.

Food and Agriculture Organization of the United Nations Statistical Database (FAOSTAT). (2022a). Annual population. https://www.fao.org/faostat/en/#data/OA Date of access: 25 July 2022.

Food and Agriculture Organization of the United Nations Statistical Database (FAOSTAT). (2022b). Crops and livestock products. https://www.fao.org/faostat/en/#data/TCL Date of access: 25 July 2022.

Fourie, H., Jones, R.K., Spaull, V.W., Daneel, M.S. & De Waele, D. (2017). Introduction. (*In* Fourie, H., Spaull, V.W., Jones, R.K., Daneel, M.S. & De Waele, D., *eds*. Nematology in South Africa: a view from the 21st century. Switzerland: Springer International Publishing. p. 1–12).

Fraser, D.L., Stander, B.A. & Steenkamp, V. (2019). Cytotoxic activity of pentachlorophenol and its active metabolites in SH-SY5Y neuroblastoma cells. *Toxicology in Vitro*, 58:118–125. https://doi.org/10.1016/j.tiv.2019.03.024

Friedmann, A.S. (2002). Atrazine inhibition of testosterone production in rat males following peripubertal exposure. *Reproductive Toxicology*, 16:275–279.

Gaaied, S., Oliveira, M., Le Bihanic, F., Cachot, J. & Banni, M. (2019). Gene expression patterns and related enzymatic activities of detoxification and oxidative stress systems in zebrafish larvae exposed to the 2,4-dichlorophenoxyacetic acid herbicide. *Chemosphere*, 224:289–297. https://doi.org/10.1016/j.chemosphere.2019.02.125

Gan, J., Papiernik, S.K., Koskinen, W.C. & Yates, S.R. (1999). Evaluation of accelerated solvent extraction (ASE) for analysis of pesticide residues in soil. *Environmental Science & Technology*, 33:3249–3253. https://doi.org/10.1021/es990145

García-Ayllón, M.S., Millán, C., Serra-Basante, C., Bataller, R. & Sáez-Valero, J. (2012). Readthrough acetylcholinesterase is increased in human liver cirrhosis. *PLoS ONE*, 7(9):e44598. https://doi.org/10.1371/journal.pone.0044598

García-Ayllón, M.S., Silveyra, M.X., Candela, A., Compañ, A., Clària, J., Jover, R., Pérez-Mateo, M., Felipo, V., Martínez, S., Galcerán, J. & Sáez-Valero, J. (2006). Changes in liver and plasma acetylcholinesterase in rats with cirrhosis induced by bile duct ligation. *Hepatology*, 43(3):444–453. https://doi.org/10.1002/hep.21071

Garcia-Reyero, N. (2018). The clandestine organs of the endocrine system. *General and Comparative Endocrinology*, 257:264–271. https://doi.org/10.1016/j.ygcen.2017.08.017

Garcimartín, A., López-Oliva, M.E., González, M.P., Sánchez-Muniz, F.J. & Benedí, J. (2017). Hydrogen peroxide modifies both activity and isoforms of acetylcholinesterase in human neuroblastoma SH-SY5Y cells. *Redox Biology*, 12:719–726. https://doi.org/10.1016/j.redox.2017.04.004

Gargouri, B., Boukholda, K., Kumar, A., Benazzouz, A., Fetoui, H., Fiebich, B.L. & Bouchard, M. (2020). Bifenthrin insecticide promotes oxidative stress and increases inflammatory mediators in human neuroblastoma cells through NF-kappaB pathway. *Toxicology in Vitro*, 65:104792. https://doi.org/10.1016/j.tiv.2020.104792

Gasnier, C., Dumont, C., Benachour, N., Clair, E., Chagnon, M.C. & Séralini, G.E. (2009). Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. *Toxicology*, 262:184–191. https://doi.org/10.1016/j.tox.2009.06.006

Ge, W., Yan, S., Wang, J., Zhu, L., Chen, A. & Wang, J. (2015). Oxidative stress and DNA damage induced by imidacloprid in zebrafish (*Danio rerio*). *Journal of Agricultural and Food Chemistry*, 63:1856–1862. https://doi.org/10.1021/jf504895h

Gea, M., Zhang, C., Tota, R., Gilardi, G., di Nardo, G. & Schilirò, T. (2022). Assessment of five pesticides as endocrine-disrupting chemicals: Effects on estrogen receptors and aromatase. *International Journal of Environmental Research and Public Health*, 19:1959. https://doi.org/10.3390/ijerph19041959

Gerber, R., Smit, N.J., van Vuren, J.H.J., Nakayama, S.M.M., Yohannes, Y.B., Ikenaka, Y., Ishizuka, M. & Wepener, V. (2015). Application of a Sediment Quality Index for the assessment and monitoring of metals and organochlorines in a premier conservation area. *Environmental Science and Pollution Research*, 22(24):19971–19989. https://doi.org/10.1007/s11356-015-5206-z

Gevao, B., Semple, K. T. & Jones, K. C. (2000). Bound pesticide residues in soils: A review. *Environmental Pollution*, 108(1):3–14. https://doi.org/10.1016/S0269-7491(99)00197-9

Ghisari, M., Long, M., Tabbo, A. & Bonefeld-Jørgensen, E.C. (2015). Effects of currently used pesticides and their mixtures on the function of thyroid hormone and aryl hydrocarbon receptor in cell culture. *Toxicology and Applied Pharmacology*, 284:292–303. https://doi.org/10.1016/j.taap.2015.02.004

Goad, R.T., Goad, J.T., Atieh, B.H. & Gupta, R.C. (2004). Carbofuran-induced endocrine disruption in adult male rats. *Toxicology Mechanisms and Methods*, 14:233–239. https://doi.org/10.1080/15376520490434476

Gomes, A., Fernandes, E. & Lima, J.L.F.C. (2005). Fluorescence probes used for detection of reactive oxygen species. *Journal of Biochemical and Biophysical Methods*, 65:45–80. https://doi.org/10.1016/j.jbbm.2005.10.003

Gomez, S. D., Bustos, P.S., Sánchez, V.G., Ortega, M. G. & Guiñazú, N. (2020). Trophoblast toxicity of the neonicotinoid insecticide acetamiprid and an acetamiprid-based formulation. *Toxicology*, 431:152363. https://doi.org/10.1016/j.tox.2020.152363

Gordan, R., Gwathmey, J.K. & Xie, L. (2015). Autonomic and endocrine control of cardiovascular function. *World Journal of Cardiology*, 7(4):204–214. https://doi.org/10.4330/wjc.v7.i4.204

Gore, A.C., Chappell, V.A., Fenton, S.E., Flaws, J.A., Nadal, A., Prins, G.S., Toppari, J. & Zoeller, R.T. (2015). EDC-2: The Endocrine Society's second scientific statement on endocrine-disrupting chemicals. *Endocrine Reviews*, 36:1–150. https://doi.org/10.1210/er.2015-1010

Grain SA. (2017). Potential export market opportunities for South African maize. https://www.grainsa.co.za/potential-export-market-opportunities-for-south-african-maize Date of access: 1 August 2022.

Grain SA. (2021). Prodcution report: area and production of white- and yellow maize. https://www.grainsa.co.za/pages/industry-reports/production-reports Date of access: 17 Jan. 2022.

Gray, B.H., Lee, L.H. & Wyman, J.F. (1985). An automated analysis for superoxide dismutase enzyme activity. *Journal of Analytical Toxicology*, 9:36–39. https://doi.org/10.1093/jat/9.1.36

Grela, E., Kozłowska, J. & Grabowiecka, A. (2018). Current methodology of MTT assay in bacteria – A review. *Acta Histochemica*, 120:303–311. https://doi.org/10.1016/j.acthis.2018.03.007

Graymore, M., Stagnitti, F. & Allinson, G. (2001). Impacts of atrazine in aquatic ecosystems. *Environmental International*, 26:483–495. https://doi.org/10.1016/S0160-4120(01)00031-9

Griboff, J., Morales, D., Bertrand, L., Bonansea, R.I., Monferrán, M.V., Asis, R., Wunderlin, D.A. & Amé, M.V. (2014). Oxidative stress response induced by atrazine in *Palaemonetes argentinus*: The protective effect of vitamin E. *Ecotoxicology and Environmental Safety*, 108:1–8. https://doi.org/10.1016/j.ecoenv.2014.06.025

Gu, X. & Manautou, J.E. (2012). Molecular mechanisms underlying chemical liver injury. *Expert Reviews in Molecular Medicine*, 14:e4. https://doi.org/10.1017/S1462399411002110

Gu, X. & Wang, X. (2021). An overview of recent analysis and detection of acetylcholine. *Analytical Biochemistry*, 632:114381. https://doi.org/10.1016/j.ab.2021.114381

Guzzella, L. & Pozzoni, F. (1999). Accelerated solvent extraction of herbicides in agricultural soil samples. *International Journal of Environmental Analytical Chemistry*, 74(1–4):123–133. https://doi.org/10.1080/03067319908031420

Halliwell, B. & Whiteman, M. (2004). Measuring reactive species and oxidative damage *in vivo* and in cell culture: how should you do it and what do the results mean? *British Journal of Pharmacology*, 142(2):231–255. https://doi.org/10.1038/sj.bjp.0705776

Hama, J.R., Kolpin, D.W., Lefevre, G.H., Hubbard, L.E., Powers, M.M. & Strobel, B.W. (2021). Exposure and Transport of alkaloids and phytoestrogens from soybeans to agricultural soils and streams in the midwestern

United States. *Environmental Science and Technology*, 55:11029–11039. https://doi.org/10.1021/acs.est.1c01477

Hankinson, O. 1995. The aryl hydrocarbon receptor complex. *Annual Review of Pharmacology and Toxicology*, 35:307–340. https://doi.org/10.1146/annurev.pa.35.040195.001515

Harada, Y., Tanaka, N., Ichikawa, M., Kamijo, Y., Sugiyama, E., Gonzalez, F.J., & Aoyama, T. (2016). PPARα-dependent cholesterol/testosterone disruption in Leydig cells mediates 2,4-dichlorophenoxyacetic acid-induced testicular toxicity in mice. *Archives of Toxicology*, 90(12):3061–3071. https://doi.org/10.1007/s00204-016-1669-z

Harju, A.V., Närhi, I., Mattsson, M., Kerminen, K., & Kontro, M.H. (2021). Organic matter causes chemical pollutant dissipation along with adsorption and microbial degradation. *Frontiers in Environmental Science*, 9:666222. https://doi.org/10.3389/fenvs.2021.666222

Harp, P.R. (2010). Dicamba. (*In* Krieger, R., *ed*. Hayes' handbook of pesticide toxicology. Amsterdam: Elsevier. p. 1849–1852). https://doi.org/10.1016/B978-0-12-374367-1.00085-9

Harry, G.J., Billingsley, M., Bruinink, A., Campbell, I.L., Classen, W., Dorman, D.C., Galli, C., Ray, D., Smith, R.A. & Tilson, H.A. (1998). *In vitro* techniques for the assessment of neurotoxicity. *Environmental Health Perspectives*, 106:131–158. https://doi.org/10.1289/ehp.98106s1131

Härtl, R., Gleinich, A. & Zimmermann, M. (2011). Dramatic increase in readthrough acetylcholinesterase in a cellular model of oxidative stress. *Journal of Neurochemistry*, 116(6):1088–1096. https://doi.org/10.1111/j.1471-4159.2010.07164.x

Hattab, S., Boughattas, I., Boussetta, H., Viarengo, A., Banni, M. & Sforzini, S. (2015). Transcriptional expression levels and biochemical markers of oxidative stress in the earthworm *Eisenia andrei* after exposure to 2,4-dichlorophenoxyacetic acid (2,4-D). *Ecotoxicology and Environmental Safety*, 122:76–82. https://doi.org/10.1016/j.ecoenv.2015.07.014

Hayes, T.B., Khoury, V., Narayan, A., Nazir, M., Parka, A., Brown, T., Adame, L., Chan, E., Buchholz, D., Stueve, T. & Gallipeau, S. (2010). Atrazine induces complete feminization and chemical castration in male African clawed frogs (*Xenopus laevis*). *Proceedings of the National Academy of Sciences of the United States of America*, 107(10):4612–4617. https://doi.org/10.1073/pnas.0909519107

He, L., He, T., Farrar, S., Ji, L., Liu, T. & Ma, X. (2017). Antioxidants maintain cellular redox homeostasis by elimination of reactive oxygen species. *Cellular Physiology and Biochemistry*, 44(2):532–553. https://doi.org/10.1159/000485089

Heindel, J.J. & Blumberg, B. (2019). Environmental obesogens: Mechanisms and controversies. *Annual Review of Pharmacology and Toxicology*, 59:4.1-4.18. https://doi.org/10.1146/annurev-pharmtox-010818-021304

Hiller-Sturmhöfel, S. & Bartke, A. (1998). The endocrine system: An overview. *Alcohol Health and Research World*, 22(3):153–164.

Hogendoorn, E.A., Hoogerbrugge, R., Baumann, R.A., Meiring, H.D., de Jong, A.P.J.M. & van Zoonen, P. (1996). Screening and analysis of polar pesticides in environmental monitoring programmes by coupled-column liquid chromatography and gas chromatography-mass spectrometry. *Journal of Chromatography A*, 754:49–60. https://doi.org/10.1016/S0021-9673(96)00376-7

Holloway, A.C., Anger, D.A., Crankshaw, D.J., Wu, M. & Foster, W.G. (2008). Atrazine-induced changes in aromatase activity in estrogen sensitive target tissues. *Journal of Applied Toxicology*, 28:260–270. https://doi.org/10.1002/jat

Holm, S. (1979). A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics*, 6(2):65–70.

Horak, I., Horn, S. & Pieters, R. (2021). Agrochemicals in freshwater systems and their potential as endocrine disrupting chemicals: A South African context. *Environmental Pollution*, 268:115718. https://doi.org/10.1016/j.envpol.2020.115718

Horn, S., Pieters, R. & Bøhn, T. (2019). A first assessment of glyphosate, 2,4-D and Cry proteins in surface water of South Africa. *South African Journal of Science*, 115(9–10):1–7. https://doi.org/10.17159/sajs.2019/5988

Horn, S., Pieters, R. & Bøhn, T. (2020). May agricultural water sources containing mixtures of agrochemicals cause hormonal disturbances? *Science of the Total Environment*, 711:134862. https://doi.org/10.1016/j.scitotenv.2019.134862

Hotchkiss, A.K., Ostby, J.S., Vandenbergh, J.G. & Gray, L.E. (2002). Androgens and environmental antiandrogens affect reproductive development and play behaviour in the Sprague-Dawley rat. *Environmental Health Perspectives*, 110:435–439. https://doi.org/10.1289/ehp.02110s3435

Huang, T., Huang, Y., Huang, Y., Yang, Y., Zhao, Y. & Martyniuk, C. J. (2020a). Toxicity assessment of the herbicide acetochlor in the human liver carcinoma (HepG2) cell line. *Chemosphere*, 243:125345. https://doi.org/10.1016/j.chemosphere.2019.125345

Huang, W.-K., Xie, C., Young, R.L., Zhao, J.-B., Ebendorff-Heidepriem, H., Jones, K.L., Rayner, C.K. & Wu, T.-Z. (2020b). Development of innovative tools for investigation of nutrient-gut interaction. *World Journal of Gastroenterology*, 26(25):3562–3576. https://doi.org/10.3748/wjg.v26.i25.3562

Huanyu, T., Jianghong, S., Wei, G., Jiawei, Z., Hui, G. & Yunhe, W. (2022). Environmental fate and toxicity of androgens: A critical review. *Environmental Research*, 214:113849. https://doi.org/10.1016/j.envres.2022.113849

Humann-Guilleminot, S., Binkowski, Ł.J., Jenni, L., Hilke, G., Glauser, G. & Helfenstein, F. (2019). A nation-wide survey of neonicotinoid insecticides in agricultural land with implications for agri-environment schemes. *Journal of Applied Ecology*, 56(7):1502–1514. https://doi.org/10.1111/1365-2664.13392

Iavicoli, I., Fontana, L. & Bergamaschi, A. (2009). The effects of metals as endocrine disruptors.In *Journal of Toxicology and Environmental Health - Part B: Critical Reviews*, 12(3):206–223. https://doi.org/10.1080/10937400902902062

Ilboudo, S., Fouche, E., Rizzati, V., Toé, A.M., Gamet-Payrastre, L. & Guissou, P. I. (2014). *In vitro* impact of five pesticides alone or in combination on human intestinal cell line Caco-2. *Toxicology Reports*, 1:474–489. https://doi.org/10.1016/j.toxrep.2014.07.008

Innovative Sustainable Remediation. (2017). Environment Quality Standards (EQS) Limit and Guideline Values for Contaminated Sites. European Union-Financed INSURE Project Report.

International Organisation for Standardisation (ISO). (2009). Biological evaluation of medical devices —Part 5: Tests for *in vitro* cytotoxicity. ISO 10993-5:2009(E).

Islam, F., Wang, J., Farooq, M.A., Khan, M.S.S., Xu, L., Zhu, J., Zhao, M., Muños, S., Li, Q.X. & Zhou, W. (2018). Potential impact of the herbicide 2,4-dichlorophenoxyacetic acid on human and ecosystems. *Environment International*, 111:332–351. https://doi.org/10.1016/j.envint.2017.10.020

Itoh, K., Kinoshita, M., Morishita, S., Chida, M. & Suyama, K. (2013). Characterization of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid-degrading fungi in Vietnamese soils. *FEMS Microbiology Ecology*, 84(1):124–132. https://doi.org/10.1111/1574-6941.12043

Jabłońska-Trypuć, A., Wołejko, E., Wydro, U. & Butarewicz, A. (2017). The impact of pesticides on oxidative stress level in human organism and their activity as an endocrine disruptor. *Journal of Environmental Science and Health - Part B Pesticides, Food Contaminants, and Agricultural Wastes*, 52(7):483–494. https://doi.org/10.1080/03601234.2017.1303322

JanakiDevi, V., Nagarani, N., YokeshBabu, M., Kumaraguru, A.K. & Ramakritinan, C.M. (2013). A study of proteotoxicity and genotoxicity induced by the pesticide and fungicide on marine invertebrate (*Donax faba*). *Chemosphere*, 90(3):1158–1166. https://doi.org/10.1016/j.chemosphere.2012.09.024

Janošek, J., Bittner, M., Hilscherová, K., Bláha, L., Giesy, J.P. & Holoubek, I. (2007). AhR-mediated and antiestrogenic activity of humic substances. *Chemosphere*, 67(6):1096–1101. https://doi.org/10.1016/j.chemosphere.2006.11.045.

Jausons-Loffreda, N., Balaguer, P., Roux, S., Fuentes, M., Pons, M. & Nicolas, J.-C. (1994). Chimeric receptors as a tool for luminescent measurement of biological activities of steroid hormones. *Journal of Bioluminescence and Chemiluminescence*, 9:217–221. https://doi.org/10.1002/bio.1170090316

Jindal, R. & Kaur, M. (2014). Acetylcholinesterase inhibition and assessment of its recovery response in some organs of *Ctenopharyngodon idellus* induced by chlorpyrifos. *International Journal of Science, Environment and Technology*, 3(2):473–480.

Jury, M.R. (2021). Spreading of the semi-arid climate across South Africa. *Journal of Water & Climate Change*, 12(8):3734–3749. https://doi.org/10.2166/wcc.2021.187

Kaltenbach, J.C. (1988). Endocrine aspects of homeostasis. *American Zoologist*, 28(2):761–773. https://doi.org/10.1093/icb/28.2.761

Kapoor, U., Srivastava, M.K., Bhardwaj, S. & Srivastava, L.P. (2010). Effect of imidacloprid on antioxidant enzymes and lipid peroxidation in female rats to derive its No Observed Effect Level (NOEL). *The Journal of Toxicological Sciences*, 35(4):577–581. https://doi.org/10.2131/jts.35.577

Karthikeyan, B.S., Ravichandran, J., Mohanraj, K., Vivek-Ananth, R.P. & Samal, A. (2019). A curated knowledgebase on endocrine disrupting chemicals and their biological systems-level perturbations. *Science of the Total Environment*, 692:281–296. https://doi.org/10.1016/j.scitotenv.2019.07.225

Kass, L., Gomez, A.L. & Altamirano, G.A. (2020). Relationship between agrochemical compounds and mammary gland development and breast cancer. *Molecular and Cellular Endocrinology*, 508:110789. https://doi.org/10.1016/j.mce.2020.110789

Kašuba, V., Milić, M., Rozgaj, R., Kopjar, N., Mladinić, M., Žunec, S., Vrdoljak, A. L., Pavičić, I., Čermak, A. M.M., Pizent, A., Lovaković, B.T. & Želježić, D. (2017). Effects of low doses of glyphosate on DNA damage, cell proliferation and oxidative stress in the HepG2 cell line. *Environmental Science and Pollution Research*, 24:19267–19281. https://doi.org/10.1007/s11356-017-9438-y

Katerji, M., Filippova, M. & Duerksen-Hughes, P. (2019). Approaches and methods to measure oxidative stress in clinical samples: research applications in the cancer field. *Oxidative Medicine and Cellular Longevity*, 2019:1279250. https://doi.org/10.1155/2019/1279250

Keita, D., Shishodia, S. & Sridhar, B.B.M. (2021). Cytotoxicity analysis of pre- and post-hurricane harvey soil samples collected from greater Houston bayous. *Ecotoxicology and Environmental Safety*, 223:112600. https://doi.org/10.1016/j.ecoenv.2021.112600

Keston, A.S. & Brandt, R. (1965). The fluorometric analysis of ultramicro quantities of hydrogen peroxide. *Analytical Biochemistry*, 11:1–5. https://doi.org/10.1016/0003-2697(65)90034-5

Khalil, A.M. (2015). Toxicological effects and oxidative stress responses in freshwater snail, *Lanistes carinatus*, following exposure to chlorpyrifos. *Ecotoxicology and Environmental Safety*, 116:137–142. https://doi.org/10.1016/j.ecoenv.2015.03.010

Khammissa, R.A.G., Ballyram, R., Wood, N. H., Lemmer, J. & Feller, L. (2016). Glucocorticosteroids in the treatment of immune mediated oral diseases. *Clinical Reviews*, 71(2):62–67. www.sada.co.za/sadJvol71No.2

Kidd, K.A., Blanchfield, P.J., Mills, K.H., Palace, V.P., Evans, R.E., Lazorchak, J.M. & Flick, R.W. (2007). Collapse of a fish population after exposure to a synthetic estrogen. *Proceedings of the National Academy of Sciences*, 104(21):8897–8901. www.pnas.orgcgidoi10.1073pnas.0609568104

Kim, H.J., Park, Y.I. & Dong, M.S. (2006). Comparison of prostate cancer cell lines for androgen receptor-mediated reporter gene assays. *Toxicology in Vitro*, 20:1159–1167. https://doi.org/10.1016/j.tiv.2006.03.003

Kimura-Kuroda, J., Komuta, Y., Kuroda, Y., Hayashi, M. & Kawano, H. (2012). Nicotine-like effects of the neonicotinoid insecticides acetamiprid and imidacloprid on cerebellar neurons from neonatal rats. *PLoS ONE*, *7*(2):e32432. https://doi.org/10.1371/journal.pone.0032432

Klein, K., Hof, D., Dombrowski, A., Schweyen, P., Dierkes, G., Ternes, T., Schulte-Oehlmann, U. & Oehlmann, J. (2021). Enhanced *in vitro* toxicity of plastic leachates after UV irradiation. *Water Research*, 199:117203. https://doi.org/10.1016/j.watres.2021.117203

Kleine, B. & Rossmanith, W.G. (2016). *Hormones and the Endocrine System*. Springer International Publishing: Switzerland. https://doi.org/10.1007/978-3-319-15060-4

Klopčič, I., Kolšek, K. & Dolenc, M.S. (2015). Glucocorticoid-like activity of propylparaben, butylparaben, diethylhexyl phthalate and tetramethrin mixtures studied in the MDA-kb2 cell line. *Toxicology Letters*, 232:376–383. https://doi.org/10.1016/j.toxlet.2014.11.019

Kojima, H., Katsura, E., Takeuchi, S., Niiyama, K. & Kobayashi, K. (2004). Screening for estrogen and androgen receptor activities in 200 pesticides by *in vitro* reporter gene assays using Chinese hamster ovary cells. *Environmental Health Perspectives*, 112(5):524–531. https://doi.org/10.1289/ehp.6649

Kojima, H., Takeuchi, S. & Nagai, T. (2010). Endocrine-disrupting potential of pesticides via nuclear receptors and aryl hydrocarbon receptor. *Journal of Health Science*, 56(4):374–386. https://doi.org/10.1248/jhs.56.374

Könemann, S., Kase, R., Simon, E., Swart, K., Buchinger, S., Schlüsener, M., Hollert, H., Escher, B.I., Werner, I., Aït-aïssa, S., Vermeirssen, E., Dulio, V., Valsecchi, S., Polesello, S., Behnisch, P., Javurkova, B., Perceval, O., Di, C., Olbrich, D., Sychrova, E., Schlichting, R., Leborgne, L., Clara, M., Scheffknecht, C., Marneffe, Y., Chalon, C., Tu, P., Danwitz, B. Von, Schwaiger, J., Isabel, M., Martín, S., Bersani, F., Hilscherov, K., Reifferscheid, G., Ternes, T., & Carere, M. (2018). Effect-based and chemical analytical methods to monitor estrogens under the European Water Framework Directive. *Trends in Analytical Chemistry*, 102:225–235. https://doi.org/10.1016/j.trac.2018.02.008

Kortenkamp, A. (2008). Low dose mixture effects of endocrine disrupters: Implications for risk assessment and epidemiology. *International Journal of Andrology*, 31(2):233–240. https://doi.org/10.1111/j.1365-2605.2007.00862.x

Kremer, E., Rompa, M. & Zygmunt, B. (2004). Extraction of acidic herbicides from soil by means of accelerated solvent extraction. *Chromatographia*, 60:S169–S174. https://doi.org/10.1365/s10337-004-0186-z

Kulik, M., Bothe, M., Kibar, G., Fuchs, A., Schöne, S., Prekovic, S., Mayayo-Peralta, I., Chung, H. R., Zwart, W., Helsen, C., Claessens, F. & Meijsing, S. H. (2021). Androgen and glucocorticoid receptor direct distinct

transcriptional programs by receptor-specific and shared DNA binding sites. *Nucleic Acids Research*, 49(7):3856–3875. https://doi.org/10.1093/nar/gkab185

Kumar, R., Zakharov, M.N., Khan, S.H., Miki, R., Jang, H., Toraldo, G., Singh, R., Bhasin, S. & Jasuja, R. (2011). The dynamic structure of the estrogen receptor. *Journal of Amino Acids*, 2011:812540. https://doi.org/10.4061/2011/812540

La Merrill, M.A., Vandenberg, L.N., Smith, M.T., Goodson, W., Browne, P., Patisaul, H.B., Guyton, K.Z., Kortenkamp, A., Cogliano, V.J., Woodruff, T.J., Gore, A.C., Korach, K.S., Gore, A.C., Zeise, L. & Zoeller, R.T. (2020). Consensus on the key characteristics of endocrine-disrupting chemicals as a basis for hazard identification. *Nature Reviews Endocrinology*, 16:45–57. https://doi.org/10.1038/s41574-019-0273-8

Lacorte, S. & Fernandez-Alba, A.R. (2006). Time of flight mass spectrometry applied to the liquid chromatographic analysis of pesticides in water and food. *Mass Spectrometry Reviews*, 25:866–880. https://doi.org/10.1002/mas.20094

Larigot, L., Juricek, L., Dairou, J. & Coumoul, X. (2018). AhR signaling pathways and regulatory functions. *Biochimie Open*, 7:1–9. https://doi.org/10.1016/j.biopen.2018.05.001

LeBel, C.P., Ischiropoulos, H. & Bondy, S.C. (1992). Evaluation of the probe 2′,7′-dichlorofluorescin as an indicator of reactive oxygen species formation and oxidative stress. *Chemical Research in Toxicology*, 5:227–231. https://doi.org/10.1021/tx00026a012

Lee, H.-R., Kim, T.-H. & Choi, K.-C. (2012). Functions and physiological roles of two types of estrogen receptors, ERα and ERβ, identified by estrogen receptor knockout mouse. *Laboratory Animal Research*, 28(2):71–76. https://doi.org/10.5625/lar.2012.28.2.71

Legler, J., van den Brink, C., Brouwer, A., Murk, A.J., van der Saag, P.T., Vethaak, A.D. & van der Burg, B. (1999). Development of a stably transfected estrogen receptor-mediated luciferase reporter gene assay in the human T47D breast cancer cell line. *Toxicological Sciences*, 48:55–66. https://doi.org/10.1093/toxsci/48.1.55

Legradi, J.B., Di Paolo, C., Kraak, M.H.S., van der Geest, H.G., Schymanski, E.L., Williams, A.J., Dingemans, M.M.L., Massei, R., Brack, W., Cousin, X., Begout, M.-L., R. van der Oost, R., Carion, A., Suarez-Ulloa, F., Silvestre, F., Escher, B.I., Engwall, M., Nilén, G., Keiter, S.H., Pollet, D., Waldmann, P., Kienle, C., Werner, I., Haigis, A.-C., Knapen, D., Vergauwen, L., Spehr, M., Schulz, W., Busch, W., Leuthold, D., Scholz, S., vom Berg, C.M., Basu, N., Murphy, C.A., Lampert, A., Kuckelkorn, J., Grummt, T. & Hollert, H. (2018). An ecotoxicological view on neurotoxicity assessment. *Environmental Sciences Europe*, 30:46. https://doi.org/10.1186/s12302-018-0173-x

Leskinen, P., Hilscherova, K., Sidlova, T., Kiviranta, H., Pessala, P., Salo, S., Verta, M. & Virta, M. (2008). Detecting AhR ligands in sediments using bioluminescent reporter yeast. *Biosensors and Bioelectronics*, *23*, 1850–1855. https://doi.org/10.1016/j.bios.2008.02.026

Leusch, F.D.L., Aneck-Hahn, N.H., Cavanagh, J.-A.E., du Pasquier, D., Hamers, T., Hebert, A., Neale, P.A., Scheurer, M., Simmons, S.O. & Schriks, M. (2018). Comparison of *in vitro* and *in vivo* bioassays to measure thyroid hormone disrupting activity in water extracts. *Chemosphere*, 191:868–875. https://doi.org/10.1016/j.chemosphere.2017.10.109

Leusch, F.D.L., Neale, P.A., Hebert, A., Scheurer, M. & Schriks, M.C.M. (2017). Analysis of the sensitivity of in vitro bioassays for androgenic, progestagenic, glucocorticoid, thyroid and estrogenic activity: Suitability for drinking and environmental waters. *Environment International*, 99:120–130. https://doi.org/10.1016/j.envint.2016.12.014

Li, J., Wang, Y., Kong, D., Wang, J., Teng, Y. & Li, N. (2015). Evaluation and characterization of anti-estrogenic and anti-androgenic activities in soil samples along the Second Songhua River, China. *Environmental Monitoring and Assessment*, 187:724. https://doi.org/10.1007/s10661-015-4933-4

Li, N., Sioutas, C., Cho, A., Schmitz, D., Misra, C., Sempf, J., Wang, M., Oberley, T., Froines, J. & Nel, A. (2003). Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. *Environmental Health Perspectives*, *111*(4):455–460. https://doi.org/10.1289/ehp.6000

Li, Y., Padoan, E. & Ajmone-Marsan, F. (2021a). Soil particle size fraction and potentially toxic elements bioaccessibility: A review. *Ecotoxicology and Environmental Safety*, 209:111806. https://doi.org/10.1016/j.ecoenv.2020.111806

Li, S., Zhao, J., Huang, R., Travers, J., Klumpp-Thomas, C., Yu, W., Mackerell, A.D., Sakamuru, S., Ooka, M., Xue, F., Sipes, N.S., Hsieh, J.H., Ryan, K., Simeonov, A., Santillo, M.F. & Xia, M. (2021b). Profiling the tox21 chemical collection for acetylcholinesterase inhibition. *Environmental Health Perspectives*, 129(4): 047008-1– 047008-12. https://doi.org/10.1289/EHP6993

Lionetto, M.G., Caricato, R., Calisi, A., Giordano, M.E. & Schettino, T. (2013). Acetylcholinesterase as a biomarker in environmental and occupational medicine: New insights and future perspectives. *BioMed Research International*, 2013:321213. https://doi.org/10.1155/2013/321213

Liu, J., Liu, G., Zhang, J., Yin, H. & Wang, R. (2012). Occurrence and risk assessment of polycyclic aromatic hydrocarbons in soil from the Tiefa coal mine district, Liaoning, China. *Journal of Environmental Monitoring*, 14(10):2634–2642. https://doi.org/10.1039/c2em30433c

López-García, J., Lehocký, M., Humpolíček, P. & Sáha, P. (2014). HaCaT keratinocytes response on antimicrobial atelocollagen substrates: Extent of cytotoxicity, cell viability and proliferation. *Journal of Functional Biomaterials*, *5*(2):43–57. https://doi.org/10.3390/jfb5020043

Lu, X.T., Ma, Y., Wang, C., Zhang, X.F., da Jin, Q. & Huang, C.J. (2012). Cytotoxicity and DNA damage of five organophosphorus pesticides mediated by oxidative stress in PC12 cells and protection by vitamin E. *Journal of Environmental Science and Health, Part B*, 47:445–454. https://doi.org/10.1080/03601234.2012.663312

Lundqvist, J., von Brömssen, C., Rosenmai, A.K., Ohlsson, Å., le Godec, T., Jonsson, O., Kreuger, J. & Oskarsson, A. (2019). Assessment of pesticides in surface water samples from Swedish agricultural areas by integrated bioanalysis and chemical analysis. *Environmental Sciences Europe*, 31:53. https://doi.org/10.1186/s12302-019-0241-x

Ma, M., Chen, C., Yang, G., Wang, Y., Wang, T., Li, Y. & Qian, Y. (2019). Combined anti-androgenic effects of mixtures of agricultural pesticides using *in vitro* and *in silico* methods. *Ecotoxicology and Environmental Safety*, 186:109652. https://doi.org/10.1016/j.ecoenv.2019.109652

Mahomed, S.I., Voyi, K.V.V., Aneck-Hahn, N.H. & de Jager, C. (2008). Oestrogenicity and chemical target analysis of water from small-sized industries in Pretoria, South Africa. *Water SA*, 34(3):357–363. https://doi.org/10.4314/wsa.v34i3.180629

Mancini, F., Woodcock, B.A. & Isaac, N.J.B. (2019). Agrochemicals in the wild: Identifying links between pesticide use and declines of nontarget organisms. *Current Opinion in Environmental Science and Health*, 11:53–58. https://doi.org/10.1016/j.coesh.2019.07.003

Mandal, A., Sarkar, B., Mandal, S., Vithanage, M., Patra, A.K. & Manna, M.C. (2020). Impact of agrochemicals on soil health. (*In* Prasad, M.N.V., *ed*. Agrochemicals detection, treatment and remediation. Oxford: Butterworth-Heinemann. p. 161–187). https://doi.org/10.1016/b978-0-08-103017-2.00007-6

Marklund, S. & Marklund, G. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry*, 47:469–474. https://doi.org/10.1111/j.1432-1033.1974.tb03714.x

Martínez, S.R., Aiassa, V., Sola, C. & Becerra, M. C. (2020). Oxidative stress response in reference and clinical *Staphylococcus aureus* strains under linezolid exposure. *Journal of Global Antimicrobial Resistance*, 22:257–262. https://doi.org/10.1016/j.jgar.2020.02.032

Martyniuk, C.J., Mehinto, A.C. & Denslow, N.D. (2020). Organochlorine pesticides: Agrochemicals with potent endocrine-disrupting properties in fish. *Molecular and Cellular Endocrinology*, 507:110764. https://doi.org/10.1016/j.mce.2020.110764

Mattia, C.J., Lebel, C.P. & Bondy, S.C. (1991). Effects of toluene and its metabolites on cerebral reactive oxygen species generation. *Biochemical Pharmacology*, 42(4):879–882. https://doi.org/10.1016/0006-2952(91)90048-A

McCarthy, T.S. (2011). The impact of acid mine drainage in South Africa. *South African Journal of Science*, 107(5/6):712 https://doi.org/10.4102/sajs.v107i5/6.712

McCord, J.M. & Fridovich, I. (1969). Superoxide dismutase - An enzymatic function for erythrocuprein (hemocuprein). *The Journal of Biological Chemistry*, 244(22):6049–6055. https://doi.org/10.1016/S0021-9258(18)63504-5

Md Meftaul, I.M., Venkateswarlu, K., Dharmarajan, R., Annamalai, P. & Megharaj, M. (2019). Pesticides in the urban environment: a potential threat that knocks at the door. *Science of the Total Environment*, 711:34612. https://doi.org/10.1016/j.scitotenv.2019.134612

Meng, C.-K. (2008). Determination of pesticides in water by SPE and LC/MS/MS in both positive and negative ion modes. Application Note. Agilent Technologies Inc. Delaware, USA. https://www.agilent.com/cs/library/applications/5989-5320EN.pdf

Mennillo, E., Cappelli, F. & Arukwe, A. (2019). Biotransformation and oxidative stress responses in rat hepatic cell-line (H4IIE) exposed to organophosphate esters (OPEs). *Toxicology and Applied Pharmacology*, 371:84–94. https://doi.org/10.1016/j.taap.2019.04.004

Mennillo, E., Krøkje, Å., Pretti, C., Meucci, V. & Arukwe, A. (2018). Biotransformation and oxidative stress responses in rat hepatic cell-line (H4IIE) exposed to racemic ketoprofen (RS-KP) and its enantiomer, dexketoprofen (S(+)-KP). *Environmental Toxicology and Pharmacology*, 59:199–207. https://doi.org/10.1016/j.etap.2018.03.018

Mesalam, A., Kong, R., Khan, I., Chowdhury, M.M.R., Choi, B.-H., Kim, S.W., Cho, K.-W., Jin, J.I. & Kong, I.-K. (2017). Effect of charcoal:dextran stripped fetal bovine serum on in vitro development of bovine embryos. *Reproductive Biology*, 17:312–319. https://doi.org/10.1016/j.repbio.2017.09.002

Mesnage, R, Benbrook, C. & Michael N. Antoniou, M.N. (2019). Insight into the confusion over surfactant co-formulants in glyphosate-based herbicides. *Food and Chemical Toxicology*, 128:137–145. https://doi.org/10.1016/j.fct.2019.03.053

Mesnage, R. & Antoniou, M.N. (2018). Ignoring adjuvant toxicity falsifies the safety profile of commercial pesticides. *Frontiers in Public Health*, 5:361. https://doi.org/10.3389/fpubh.2017.00361

Mesnage, R., Biserni, M., Genkova, D., Wesolowski, L. & Antoniou, M.N. (2018). Evaluation of neonicotinoid insecticides for oestrogenic, thyroidogenic and adipogenic activity reveals imidacloprid causes lipid accumulation. *Journal of Applied Toxicology*, 38:1483–1491. https://doi.org/10.1002/jat.3651

Mesnage, R., Phedonos, A., Biserni, M., Arno, M., Balu, S., Corton, J.C., Ugarte, R. & Antoniou, M.N. (2017). Evaluation of estrogen receptor alpha activation by glyphosate-based herbicide constituents. *Food and Chemical Toxicology*, 108:30–42. https://doi.org/10.1016/j.fct.2017.07.025

Mesnage, R., Defarge, N., Spiroux de Vendômois, J. & Gilles-Eric Séralini, G.-E. (2014). Major pesticides are more toxic to human cells than their declared active principles. *BioMed Research International*, 2014:1–8. https://doi.org/10.1155/2014/179691

Meza, I., Rezaei, E.E., Siebert, S., Ghazaryan, G., Nouri, H., Dubovyk, O., Gerdener, H., Herbert, C., Kusche, J., Popat, E., Rhyner, J., Jordaan, A., Walz, Y. & Hagenlocher, M. (2021). Drivers, spatial patterns, and implications for drought risk management *Science of the Total Environment*, 799:149505. https://doi.org/10.1016/j.scitotenv.2021.149505

Miller, J. & Miller, J.C. (2018). Statistics and chemometrics for analytical chemistry. New Jersey: Prentice Hall.

Misaki, K., Suzuki, M., Nakamura, M., Handa, H., Iida, M., Kato, T., Matsui, S. & Matsuda, T. (2008). Aryl hydrocarbon receptor and estrogen receptor ligand activity of organic extracts from road dust and diesel exhaust particulates. *Archives of Environmental Contamination and Toxicology*, 55(2):199–209. https://doi.org/10.1007/s00244-007-9110-5.

Mitra, J. & Raghu, K. (1998). Pesticides-non target plants interactions: An overview. *Archives of Agronomy and Soil Science*, 43(6):445–500. https://doi.org/10.1080/03650349809366059

Mnguni, E.S. (2022). Pollution of the Vaal River system in South Africa: A case study. *WIT Transactions on Ecology and the Environment: Waste Management and Environmental Impact XI*, 257:17–26. https://doi.org/10.2495/WMEI220021

Moghaddam, N. S., Zakaria, M. P., Omar, D. & Sijam, K. (2012). Extraction efficiency and HPLC determination of imidacloprid in soil. *Soil and Sediment Contamination*, 21:985–995. https://doi.org/10.1080/15320383.2012.712071

Morgan, H.E., Dillaway, D. & Edwards, T.M. (2014). Estrogenicity of soybeans (*Glycine max*) varies by plant organ and developmental stage. *Endocrine Disruptors*, 2:e28490-1-e28490-8. https://doi.org/10.4161/endo.28490

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, 65:55–63. https://doi.org/10.1039/C6RA17788C

Mossa, A.-T.H., Swelam, E.S. & Mohafrash, S.M.M. (2015). Sub-chronic exposure to fipronil induced oxidative stress, biochemical and histopathological changes in the liver and kidney of male albino rats. *Toxicology Reports*, 2:775–784. https://doi.org/10.1016/j.toxrep.2015.02.009

Mostrom, M. & Evans, T.J. (2011). Phytoestrogens. (*In* Ramesh, R.C., *ed*. Reproductive and developmental toxicology. Massachusetts: Aceramic Press. p. 707–722). https://doi.org/10.1016/B978-0-12-382032-7.10052-9

Mountassif, D., Kabine, M., Karima Mounchid, Mounaji, K., Latruffe, N. & Kebbaj, M.H.S.E. (2008). Biochemical and histological alterations of cellular metabolism from jerboa (*Jaculus orientalis*) by 2,4-dichlorophenoxyacetic acid: Effects on D-3-hydroxybutyrate dehydrogenase. *Pesticide Biochemistry and Physiology*, 90(2):87–96. https://doi.org/10.1016/j.pestbp.2007.08.001

Murate, T. & Kaneda, T. (1989). Treatment of fetal bovine serum with activated charcoal enhances spontaneous differentiation of murine erythroleukemia cells. *Leukemia Research*, 13(3):227–231. https://doi.org/10.1016/0145-2126(89)90016-7

Mutengwe, M.T., Aneck-Hahn, N.H., Korsten, L., van Zijl, M.C. & de Jager, C. (2016). Pesticide residues and estrogenic activity in fruit and vegetables sampled from major fresh produce markets in South Africa. *Food Additives and Contaminants: Part A*, 33: 95–104. https://doi.org/10.1080/19440049.2015.1103905

Navarro, L., Camacho, R., López, J.E. & Saldarriaga, J.F. (2021). Assessment of the potential risk of leaching pesticides in agricultural soils: study case Tibasosa, Boyacá, Colombia. *Heliyon*, 7:e08301. https://doi.org/10.1016/j.heliyon.2021.e08301

Neale, P.A. & Leusch, F.D.L. (2015). Considerations when assessing antagonism *in vitro*: Why standardizing the agonist concentration matters. *Chemosphere*, 135:20–23. https://doi.org/10.1016/j.chemosphere.2015.03.054

Neale, P.A., Ait-aissa, S., Brack, W., Creusot, N., Denison, M.S., Deutschmann, B., Hilscherova, K., Hollert, H., Krauss, M., Novak, J., Schulze, T., Seiler, T.-B., Serra, H., Shao, Y. & Escher, B.I. (2015). Linking *in vitro* effects and detected oganic micropollutants in surface water using mixture-toxicity modeling. *Environmental Science & Technology*, 49:14614–14624. https://doi.org/10.1021/acs.est.5b04083

Neale, P.A., Braun, G., Brack, W., Carmona, E., Gunold, R., König, M., Krauss, M., Liebmann, L., Liess, M., Link, M., Schäfer, R.B., Schlichting, R., Schreiner, V.C., Schulze, T., Vormeier, P., Weisner, O. & Escher, B.I. (2020). Assessing the mixture effects in *in vitro* bioassays of chemicals occurring in small agricultural streams during rain events. *Environmental Science & Technology*, 54:8280–8290. https://doi.org/10.1021/acs.est.0c02235

Neale, P.A., Escher, B.I. & Leusch, F.D.L. (2015). Understanding the implications of dissolved organic carbon when assessing antagonism *in vitro*: An example with an estrogen receptor assay. *Chemosphere*, 135:341–346. https://doi.org/10.1016/j.chemosphere.2015.04.084

Newman, M.C. (2015). Fundamentals of ecotoxicology - the science of pollution. Boca Raton: CRC Press.

Nguyen, L.P. & Bradfield, C.A. (2008). The search for endogenous activators of the aryl hydrocarbon receptor. *Chemical Research in Toxicology*, 21(1):102–116. https://doi.org/10.1021/tx7001965.

Nimura, A., Muneta, T., Otabe, K., Koga, H., Ju, Y.J., Mochizuki, T., Suzuki, K. & Sekiya, I. (2010). Analysis of human synovial and bone marrow mesenchymal stem cells in relation to heat-inactivation of autologous and fetal bovine serums. *BMC Musculoskeletal Disorders*, 11:208. https://doi.org/10.1186/1471-2474-11-208

Nnadi, J.U., Dimelu, I.N., Nwani, S.I., Madu, J.C., Atama, C.I., Attamah, G.N., Okwor, J.I. & Nwani, C.D. (2018). Biometric variations and oxidative stress responses in juvenile *Clarias gariepinus* exposed to Termex®. *African Journal of Aquatic Science*, 43(1):27–34. https://doi.org/10.2989/16085914.2018.1445615

Njattuvetty Chandran, N., Fojtova, D., Blahova, L., Rozmankova, E. & Blaha, L. (2018). Acute and (sub)chronic toxicity of the neonicotinoid imidacloprid on *Chironomus riparius*. *Chemosphere*, 209:568–577. https://doi.org/10.1016/j.chemosphere.2018.06.102

Nuss, E.T. & Tanumihardjo, S.A. (2010). Maize: a paramount staple crop in the context of global nutrition. *Comprehensive Reviews in Food Science and Food Safety*, 9:417–436. https://doi.org/10.1111/j.1541-4337.2010.00117.x

Nwani, C.D., Nagpure, N.S., Kumar, R., Kushwaha, B. & Lakra, W.S. (2013). DNA damage and oxidative stress modulatory effects of glyphosate-based herbicide in freshwater fish, *Channa punctatus*. *Environmental Toxicology and Pharmacology*, 36:539–547. https://doi.org/10.1016/j.etap.2013.06.001

Odukkathil, G. & Vasudevan, N. (2013). Toxicity and bioremediation of pesticides in agricultural soil. *Reviews in Environmental Science and Biotechnology*, 12:421–444. https://doi.org/10.1007/s11157-013-9320-4

Ohkawa, H., Ohishi, N. & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, 95:351–358. https://doi.org/10.1016/0003-2697(79)90738-3

Ohtake, F., Fujii-Kuriyama, Y., & Kato, S. (2009). AhR acts as an E3 ubiquitin ligase to modulate steroid receptor functions. Biohemical Pharmacology, 77:474–484. https://doi.org/10.1016/j.bcp.2008.08.034

Ohtake, F., Takeyama, K., Matsumoto, T., Kitagawa, H., Yamamoto, Y., Nohara, K., Tohyama, C., Krust, A., Mimura, J., Chambon, P., Yanagisawa, J., Fujii-Kuriyama, Y. & Kato, S. (2003). Modulation of oestrogen receptor signalling by association with the activated dioxin receptor. *Nature*, 423:545–550. https://doi.org/10.1038/nature01606

Ojha, A., Yaduvanshi, S.K. & Srivastava, N. (2011). Effect of combined exposure of commonly used organophosphate pesticides on lipid peroxidation and antioxidant enzymes in rat tissues. *Pesticide Biochemistry and Physiology*, 99(2):148–156. https://doi.org/10.1016/j.pestbp.2010.11.011

Okubo, T., Yokoyama, Y., Kano, K., Soya, Y. & Kano, I. (2004). Estimation of estrogenic and antiestrogenic activities of selected pesticides by MCF-7 cell proliferation assay. *Archives of Environmental Contamination and Toxicology*, 46:445–453. https://doi.org/10.1007/s00244-003-3017-6

Omiecinski, C.J., Vanden Heuvel, J.P., Perdew, G.H. & Peters, J.M. (2011). Xenobiotic metabolism, disposition, and regulation by receptors: From biochemical phenomenon to predictors of major toxicities. *Toxicological Sciences*, 120(S1):S49–S75. https://doi.org/10.1093/toxsci/kfq338

Onder, S., Schopfer, L.M., Jiang, W., Tacal, O. & Lockridge, O. (2022). Butyrylcholinesterase in SH-SY5Y human neuroblastoma cells. *NeuroToxicology*, *90*, 1–9. https://doi.org/10.1016/j.neuro.2022.02.006

Orton, F., Säfholm, M., Jansson, E., Carlsson, Y., Eriksson, A., Fick, J., Uren Webster, T., McMillan, T., Leishman, M., Verbruggen, B., Economou, T., Tyler, C.R. & Berg, C. (2018). Exposure to an anti-androgenic herbicide negatively impacts reproductive physiology and fertility in *Xenopus tropicalis*. *Scientific Reports*, 8:9124. https://doi.org/10.1038/s41598-018-27161-2

Oseland, E., Bish, M., Steckel, L. & Bradley, K. (2020). Identification of environmental factors that influence the likelihood of off-target movement of dicamba. *Pest Management Science*, 76(9):3282–3291. https://doi.org/10.1002/ps.5887

Ozcan Oruc, E., Sevgiler, Y. & Uner, N. (2004). Tissue-specific oxidative stress responses in fish exposed to 2,4-D and azinphosmethyl. *Comparative Biochemistry and Physiology - C Toxicology and Pharmacology*, 137(1):43–51. https://doi.org/10.1016/j.cca.2003.11.006

Paithankar, J. G., Saini, S., Dwivedi, S., Sharma, A., & Chowdhuri, D. K. (2021). Heavy metal associated health hazards: An interplay of oxidative stress and signal transduction. *Chemosphere*, 262:128350. https://doi.org/10.1016/j.chemosphere.2020.128350

Pala, A. (2019). The effect of a glyphosate-based herbicide on acetylcholinesterase (AChE) activity, oxidative stress, and antioxidant status in freshwater amphipod: *Gammarus pulex* (Crustacean). *Environmental Science and Pollution Research*, 26:36869–36877. https://doi.org/10.1007/s11356-019-06804-5

Pathak, V.M., Verma, V.K., Rawat, B.S., Kaur, B., Babu, N., Sharma, A., Dewali, S., Yadav, M., Kumari, R., Singh, S., Mohapatra, A., Pandey, V., Rana, N. & Cunill, J.M. (2022). Current status of pesticide effects on environment, human health and it's eco-friendly management as bioremediation: A comprehensive review. *Frontiers in Microbiology*, 13:962619. https://doi.org/10.3389/fmicb.2022.962619

Pati, S.G., Panda, F., Samanta, L. & Paital, B. (2022). Spatio-temporal changes in oxidative stress physiology parameters in apple snail *Pila globosa* as a function of soil Mg, Ca, organic carbon and aquatic physico-chemical factors. *Environmental Geochemistry and Health*. https://doi.org/10.1007/s10653-022-01376-9

Patpan, N., Banjerdpongchai, R., Tantiworawit, A., Poofery, J., Komonrit, P., Fanhchaksai Rattanathammethee, K. T., Chai-Adisaksopha, C., Rattarittamrong, E., Norasetthada, L. & Charoenkwan, P. (2019). The effect of transfusion-dependent thalassemia patient's serum on peripheral blood mononuclear cell viability. *Journal of Cell Death*, 12:1–8. https://doi.org/10.1177/1179066018823534

Pereira Moreira, M., Silva, L.M. & Martins, W.K. (2009). The role of GADD45A in resistance to oxidative stress-mediated cell death in human colon tumor cell lines. *Applied Cancer Research*, 29(4):179–184.

Pérez-Aguilar, B., Vidal, C.J., Palomec, G., García-Dolores, F., Gutiérrez-Ruiz, M.C., Bucio, L., Gómez-Olivares, J.L. & Gómez-Quiroz, L.E. (2015). Acetylcholinesterase is associated with a decrease in cell proliferation of hepatocellular carcinoma cells. *Biochimica et Biophysica Acta - Molecular Basis of Disease*, 1852(7):1380–1387. https://doi.org/10.1016/j.bbadis.2015.04.003

Peterson, M.A., McMaster, S.A., Riechers, D.E., Skelton, J. & Stahlman, P.W. (2016). 2,4-D past, present, and future: A Review. *Weed Technology*, 30(2):303–345. https://doi.org/10.1614/wt-d-15-00131.1

Phaniendra, A., Jestadi, D.B. & Periyasamy, L. (2015). Free radicals: properties, sources, targets, and their implication in various diseases. *Indian Journal of Clinical Biochemistry*, 30(1):11–26. https://doi.org/10.1007/s12291-014-0446-0

Pheiffer, W., Wolmarans, N.J., Gerber, R., Yohannes, Y.B., Ikenaka, Y., Ishizuka, M., Smit, N.J., Wepener, V. & Pieters, R. (2018). Fish consumption from urban impoundments: What are the health risks associated with DDTs and other organochlorine pesticides in fish to township residents of a major inland city. *Science of the Total Environment*, 628–629:517–527. https://doi.org/10.1016/j.scitotenv.2018.02.075

Phele, M.J., Ejidike, I.P., & Mtunzi, F.M. (2020). Organochlorine pesticides assessment in sediment samples from Vaal River by accelerated solvent extraction technique. *Rasayan Journal of Chemistry*, 13(4):2150–2160. https://doi.org/10.31788/RJC.2020.1345905

Pickett, M.A., Dush, M.K. & Nascone-Yoder, N.M. (2017). Acetylcholinesterase plays a non-neuronal, non-esterase role in organogenesis. *Development*, 144:2764–2770. https://doi.org/10.1242/dev.149831

Pimentel, D. (1995). Amounts of pesticides reaching target pests: environmental impacts and ethics. *Journal of Agricultural and Environmental Ethics*, 8(1):17–29. https://doi.org/10.1007/BF02286399

Pimentel, D. & Levitan, L. (1986). Pesticides: amounts applied and amounts reaching pests. *BioScience*, 36(2):86–91. https://doi.org/10.2307/1310108

Pisoschi, A.M. & Pop, A. (2015). The role of antioxidants in the chemistry of oxidative stress: A review. *European Journal of Medicinal Chemistry*, 97:55–74. https://doi.org/10.1016/j.ejmech.2015.04.040

Pisoschi, A.M., Pop, A., Iordache, F., Stanca, L., Predoi, G. & Serban, A.I. (2021). Oxidative stress mitigation by antioxidants - An overview on their chemistry and influences on health status. *European Journal of Medicinal Chemistry*, 209:112891. https://doi.org/10.1016/j.ejmech.2020.112891

Pojana, G., Gomiero, A., Jonkers, N. & Marcomini, A. (2007). Natural and synthetic endocrine disrupting compounds (EDCs) in water, sediment and biota of a coastal lagoon. *Environment International*, 33:929–936. https://doi.org/10.1016/j.envint.2007.05.003

Pook, C. & Gritcan, I. (2019). Validation and application of a modified QuEChERS method for extracting neonicotinoid residues from New Zealand maize field soil reveals their persistence at nominally hazardous concentrations. *Environmental Pollution*, 255:113075. https://doi.org/10.1016/j.envpol.2019.113075

Prinsloo, S., Pieters, R. & Bezuidenhout, C.C. (2013). A cell viability assay to determine the cytotoxic effects of water contaminated by microbes. *South African Journal of Science*, 109(7/8):0069. https://doi.org/10.1590/sajs.2013/20120069

Qi, S., Wang, D., Zhu, L., Teng, M., Wang, C., Xue, X. & Wu, L. (2018). Neonicotinoid insecticides imidacloprid, guadipyr, and cycloxaprid induce acute oxidative stress in *Daphnia magna*. *Ecotoxicology and Environmental Safety*, 148:352–358. https://doi.org/10.1016/j.ecoenv.2017.10.042

Quinn, D.M. (1987). Acetylcholinesterase: Enzyme structure, reaction dynamics, and virtual transition states. *Chemical Reviews*, 87:955–979. https://doi.org/10.1021/cr00081a005

Quinn, L.P., de Vos, B.J., Fernandes-Whaley, M., Roos, C., Bouwman, H., Kylin, H., Pieters, R. & van den Berg, J. (2011). Pesticide use in South Africa: one of the largest importers of pesticides in Africa. (*In* Stoytcheva, M., *ed*. Pesticides in the modern world. London: IntechOpen. p. 49–96). https://doi.org/10.5772/16995

Raffa, C.M. & Chiampo, F. (2021). Bioremediation of agricultural soils polluted with pesticides: A review. *Bioengineering*, 8:92. https://doi.org/10.3390/bioengineering8070092

Ray, P.D., Huang, B.W. & Tsuji, Y. (2012). Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signalling. *Cellular Signalling*, 24(5):981–990. https://doi.org/10.1016/j.cellsig.2012.01.008

Readman, J.W., Albanis, T.A., Barcelo, D., Galassi, S., Tronczynski, J. & Gabrielides, G.E. (1993). Herbicide contamination of mediterranean estuarine waters: Results from a MED POL pilot survey. *Marine Pollution Bulletin*, *26*(11), 613–619. https://doi.org/10.1016/0025-326X(93)90500-J

Revollo, J.R. & Cidlowski, J.A. (2009). Mechanisms generating diversity in glucocorticoid receptor signaling. *Annals of the New York Academy of Sciences*, 1179:167–178. https://doi.org/10.1111/j.1749-6632.2009.04986.x

Rhen, T. & Cidlowski, J.A. (2005). Antiinflammatory action of glucocorticoids — New mechanisms for old drugs. *New England Journal of Medicine*, 353(16):1711–1723. https://doi.org/10.1056/NEJMra050541

Richardson, N., Gordon, A.K., Muller, W., Pletschke, B. & Whitfield, A. (2010). The use of liver histopathology, lipid peroxidation and acetylcholinesterase assays as biomarkers of contaminant-induced stress in the Cape stumpnose, *Rhabdosargus holubi* (Teleostei: Sparidae), from selected South African estuaries. *Water SA*, 36(4):407–416. https://doi.org/10.4314/wsa.v36i4.58409

Richter, B.E., Jones, B.A., Ezzell, J.L., Porter, N.L., Avdalovic, N. & Pohl, C. (1996). Accelerated solvent extraction: A technique for sample preparation. *Analytical Chemistry*, 68(6):1033–1039. https://doi.org/10.1021/ac9508199

Riedo, J., Wettstein, F.E., Rosch, A., Herzog, C., Banerjee, S., Buchi, L., Charles, R., Wachter, D., Martin-Laurent, F., Bucheli, T.D., Walder, F. & van der Heijden, M.G.A. (2021). Widespread occurrence of pesticides in organically managed agricultural soils-The ghost of a conventional agricultural past? *Environmental Science and Technology*, 55(5):2919–2928. https://doi.org/10.1021/acs.est.0c06405

Rimayi, C., Odusanya, D., Weiss, J.M., de Boer, J. & Chimuka, L. (2018). Seasonal variation of chloro-s-triazines in the Hartbeespoort Dam catchment, South Africa. *Science of the Total Environment*, 613–614: 472–482. https://doi.org/10.1016/j.scitotenv.2017.09.119

Rimmer, D.L. & Smith, A.M. (2009). Antioxidants in soil organic matter and in associated plant materials. *European Journal of Soil Science*, 60(2):170–175. https://doi.org/10.1111/j.1365-2389.2008.01099.x

Riter, L.S., Pai, N., Vieira, B.C., Macinnes, A., Reiss, R., Hapeman, C.J. & Kruger, G.R. (2021). Conversations about the future of dicamba: the science behind off-target movement. *Journal of Agricultural and Food Chemistry*, 69:14435–14444. https://doi.org/10.1021/acs.jafc.1c05589

Ritter, W.F., Chirnside, A.E.M. & Scarborough, R.W. (1996). Leaching of dicamba in a coastal plain soil. *Journal of Environmental Science and Health - Part A Environmental Science and Engineering and Toxic and Hazardous Substance Control*, A31(3):505–517. https://doi.org/10.1080/10934529609376371

Roberge, M., Hakk, H. & Larsen, G. (2004). Atrazine is a competitive inhibitor of phosphodiesterase but does not affect the estrogen receptor. *Toxicology Letters*, 154:61–68. https://doi.org/10.1016/j.toxlet.2004.07.005

Röhrdanz, E., Ohler, S., Tran-Thi, Q.-H. & Kahl, R. (2002). The phytoestrogen daidzein affects the antioxidant enzyme system of rat hepatoma H4IIE cells. *Journal of Nutrition*, 132:370–375. https://doi.org/10.1093/jn/132.3.370

Romero, A., Ramos, E., Castellano, V., Martínez, M.A., Ares, I., Martínez, M., Martínez-Larrañaga, M.R. & Anadón, A. (2012). Cytotoxicity induced by deltamethrin and its metabolites in SH-SY5Y cells can be differentially prevented by selected antioxidants. *Toxicology in Vitro*, 26(6):823–830. https://doi.org/10.1016/j.tiv.2012.05.004

Roth, E.F. & Gilbert, H.S. (1984). The pyrogallol assay for superoxide dismutase: Absence of a glutathione artifact. *Analytical Biochemistry*, 137:50–53. https://doi.org/10.1016/0003-2697(84)90344-0

Ryu, B., Son, M.-Y., Jung, K.B., Kim, U., Kim, J., Kwon, O., Son, Y.S., Jung, C.-R., Park, J.-H. & Kim, C.-Y. (2021). Next-generation intestinal toxicity model of human embryonic stem cell-derived enterocyte-like cells. *Frontiers in Veterinary Science*, 8:587659. https://doi.org/10.3389/fvets.2021.587659

Sandoval-Herrera, N., Mena, F., Espinoza, M. & Romero, A. (2019). Neurotoxicity of organophosphate pesticides could reduce the ability of fish to escape predation under low doses of exposure. *Scientific Reports*, 9:10530. https://doi.org/10.1038/s41598-019-46804-6

Sanfeliu, C., Sebastia Á.J. & Kim, S.U. (2001). Methylmercury neurotoxicity in cultures of human neurons, astrocytes, neuroblastoma cells. *NeuroToxicology*, 22:317–327. https://doi.org/10.1016/S0161-813X(01)00015-8

Santillo, M.F. & Liu, Y. (2015). A fluorescence assay for measuring acetylcholinesterase activity in rat blood and a human neuroblastoma cell line (SH-SY5Y). *Journal of Pharmacological and Toxicological Methods*, 76:15–22. https://doi.org/10.1016/j.vascn.2015.07.002

Santovito, G., Piccinni, E., Cassini, A., Irato, P. & Albergoni, V. (2005). Antioxidant responses of the Mediterranean mussel, *Mytilus galloprovincialis*, to environmental variability of dissolved oxygen. *Comparative Biochemistry and Physiology - C Toxicology and Pharmacology*, 140(3–4):321–329. https://doi.org/10.1016/j.cca.2005.02.015

Sass, J.B. & Colangelo, A. (2006). European Union bans atrazine, while the United States negotiates continued use. *International Journal of Occupational and Environmental Health*, 12(3):260–267. https://doi.org/10.1179/oeh.2006.12.3.260

Schaafsma, A., Limay-Rios, V., Baute, T., Smith, J. & Xue, Y. (2015). Neonicotinoid insecticide residues in surface water and soil associated with commercial maize (corn) fields in Southwestern Ontario. *PLoS ONE*, 10(2):e0118139. https://doi.org/10.1371/journal.pone.0118139

Schmidt, M., Deschner, E.E., Thaler, H.T., Clements, L. & Good, R.A. (1977). Gastrointestinal cancer studies in the human to nude mouse heterotransplant system. *Gastroenterology*, 72(5):829–837. https://doi.org/10.1016/S0016-5085(77)80195-9

Schoeman, C., Mashiane, M., Dlamini, M. & Okonkwo, O. J. (2015). Quantification of selected antiretroviral drugs in a wastewater treatment works in South Africa using GC-TOFMS. *Journal of Chromatography & Separation Techniques*, 6:4. https://doi.org/10.4172/2157-7064.1000272

Schoenborn, A., Kunz, P. & Koster, M. (2015). Estrogenic activity in drainage water: a field study on a Swiss cattle pasture. *Environmental Sciences Europe*, 27:17. https://doi.org/10.1186/s12302-015-0047-4

Schumacher, B.A. (2002). Methods for the determination of total organic carbon (TOC) in soils and sediments. United States Environmental Protection Agency Environmental Sciences Division National Exposure Research Laboratory, NCEA-C 128(EMASC-001):1–23.

Semple, K.T., Doick, K.J., Jones, K.C., Burauel, P., Craven, A. & Harms, H. (2004). Defining bioavailability and bioaccessibility of contaminated soil and sediment is complicated. *Environmental Science and Technology*, 23(12):229A–231A. https://doi.org/10.1021/es040548w

Semren, T.Ž., Žunec, S. & Pizent, A. (2018). Oxidative stress in triazine pesticide toxicity: a review of the main biomarker findings. *Archives of Industrial Hygiene and Toxicology*, 69(2):109–125. https://doi.org/10.2478/aiht-2018-69-3118

Serdar, O. (2019). The effect of dimethoate pesticide on some biochemical biomarkers in *Gammarus pulex*. *Environmental Science and Pollution Research*, 26:21905–21914. https://doi.org/10.1007/s11356-019-04629-w

Severi, I., Abbatelli, S., Perugini, J., di Mercurio, E., Senzacqua, M., & Giordano, A. (2022). Butyrylcholinesterase distribution in the mouse gastrointestinal tract: An immunohistochemical study. *Journal of Anatomy*, 00:1–12. https://doi.org/10.1111/joa.13754

Sharbidre, A.A., Metkari, V. & Patode, P. (2011). Effect of diazinon on acetylcholinesterase activity and lipid peroxidation of *Poecilia reticulata*. *Research Journal of Environmental Toxicology*, 5(2):152–161. https://doi.org/10.3923/rjet.2011.152.161

Sharma, N. & Rayamajhi, M. (2022). Different aspects of weed management in maize (*Zea mays* L.): a brief review. *Advances in Agriculture*, 2022:7960175. https://doi.org/10.1155/2022/7960175

Shen, X., Chang, H., Sun, Y. & Wan, Y. (2020). Determination and occurrence of natural and synthetic glucocorticoids in surface waters. *Environment International*, 134:105278. https://doi.org/10.1016/j.envint.2019.105278

Sim, K.S., Park, S., Seo, H., Lee, S.-H., Lee, H.-S., Park, Y. & Kim, J. H. (2022). Comparative study of estrogenic activities of phytoestrogens using OECD *in vitro* and *in vivo* testing methods. *Toxicology and Applied Pharmacology*, 434:115815. https://doi.org/10.1016/j.taap.2021.115815

Simon-Delso, N., Amaral-Rogers, V., Belzunces, L.P., Bonmatin, J.M., Chagnon, M., Downs, C., Furlan, L., Gibbons, D.W., Giorio, C., Girolami, V., Goulson, D., Kreutzweiser, D.P., Krupke, C H., Liess, M., Long, E., Mcfield, M., Mineau, P., Mitchell, E.A., Morrissey, C.A., Noome, D.A., Pisa, L., Settele, J., Stark, J.D., Tapparo, A., Van Dyck, H., Van Praagh, J., van der Sluijs, J.P., Whitehorn, P.R.. & Wiemers, M. (2015). Systemic insecticides (neonicotinoids and fipronil): Trends, uses, mode of action and metabolites. *Environmental Science and Pollution Research*, 22:5–34. https://doi.org/10.1007/s11356-014-3470-y

Simpson, G.B., Badenhorst, J., Jewitt, G.P.W., Berchner, M. & Davies, E. (2019). Competition for land: The water-energy-food nexus and coal mining in Mpumalanga province, South Africa. *Frontiers in Environmental Science*, 7:86. https://doi.org/10.3389/fenvs.2019.00086

Singh, S., Tiwari, R.K. & Pandey, R.S. (2020). An insight into the impact of triazophos and deltamethrin pesticides as individual and in combination on oxidative stress and histopathological alterations in *Eudrilus eugeniae*. *Chemistry and Ecology*, 36(2):155–173. https://doi.org/10.1080/02757540.2019.1699536

Skanda, S. & Vijayakumar, B.S. (2021). Antioxidant and anti-inflammatory metabolites of a soil-derived fungus *Aspergillus arcoverdensis* SSSIHL-01. *Current Microbiology*, 78(4):1317–1323. https://doi.org/10.1007/s00284-021-02401-3

Sohoni, P. & Sumpter, J.P. (1998). Several environmental oestrogens are also anti-androgens. *Journal of Endocrinology*, 158:327–339. https://doi.org/10.1677/joe.0.1580327

Song, Y. (2014). Insight into the mode of action of 2,4-dichlorophenoxyacetic acid (2,4-D) as an herbicide. *Journal of Integrative Plant Biology*, 56(2):106–113. https://doi.org/10.1111/jipb.12131

Sonneveld, E., Riteco, J.A.C., Jansen, H.J., Pieterse, B., Brouwer, A., Schoonen, W.G. & van der Burg, B. (2006). Comparison of *in vitro* and *in vivo* screening models for androgenic and estrogenic activities. *Toxicological Sciences*, 89(1):173–187. https://doi.org/10.1093/toxsci/kfj009

Soto, A.M., Maffini, M.V., Schaeberle, C.M. & Sonnenschein, C. (2006). Strengths and weaknesses of *in vitro* assays for estrogenic and androgenic activity. *Best Practice and Research: Clinical Endocrinology and Metabolism*, 20(1):15–33. https://doi.org/10.1016/j.beem.2005.09.001

Sparks, C., Marnewick, J., Toefy, R., Snyman, R. & Odendaal, J. (2019). Baseline levels of antioxidant activities in *Mytilus galloprovincialis* along the coast of Cape Town, South Africa. *Marine Pollution Bulletin*, 140:287–293. https://doi.org/10.1016/j.marpolbul.2019.01.059

Stachniuk, A. & Fornal, E. (2016). Liquid chromatography-mass spectrometry in the analysis of pesticide residues in food. *Food Analytical Methods*, 9:1654–1665. https://doi.org/10.1007/s12161-015-0342-0

Statistics South Africa (SA). (2020). Stats SA releases Census of Commercial Agriculture 2017 Report. https://www.statssa.gov.za/?p=13144 Date of access: 22 July 2022.

Stavreva, D.A., George, A.A., Klausmeyer, P., Varticovski, L., Sack, D., Voss, T. C., Schiltz, R.L., Blazer, V.S., Iwanowicz, L.R. & Hager, G.L. (2012). Prevalent glucocorticoid and androgen activity in US water sources. *Scientific Reports*, 2:937. https://doi.org/10.1038/srep00937

Stejskalova, L., Dvorak, Z. & Pavek, P. (2011). Endogenous and exogenous ligands of aryl hydrocarbon receptor: current state of art. *Current Drug Metabolism*, 12:198–212. https://doi.org/10.2174/138920011795016818

Stoyanova, S., Georgieva, E., Velcheva, I., Iliev, I., Vasileva, T., Bivolarski, V., Tomov, S., Nyeste, K., Antal, L. & Yancheva, V. (2020). Multi-Biomarker assessment in common carp (*Cyprinus carpio*, Linnaeus 1758) liver after acute chlorpyrifos exposure. *Water*, 12(6):1837. https://doi.org/10.3390/w12061837

Streets, D.G., Devane, M.K., Lu, Z., Bond, T.C., Sunderland, E.M. & Jacob, D.J. (2011). All-time releases of mercury to the atmosphere from human activities. *Environmental Science and Technology*, *45*(24): 10485–10491. https://doi.org/10.1021/es202765m

Strober, W. (2015). Trypan blue exclusion test of cell viability. *Current Protocols in Immunology*, 111(1):A3.B.1-A3.B.3. https://doi.org/10.1002/0471142735.ima03bs111

Sturm, A., Radau, T.S., Hahn, T. & Schulz, R. (2007). Inhibition of rainbow trout acetylcholinesterase by aqueous and suspended particle-associated organophosphorous insecticides. *Chemosphere*, 68:605–612. https://doi.org/10.1016/j.chemosphere.2007.02.056

Sule, R.O., Condon, L. & Gomes, A. V. (2022). A common feature of pesticides: oxidative stress—the role of oxidative stress in pesticide-induced toxicity. *Oxidative Medicine and Cellular Longevity*, 2022:5563759. https://doi.org/10.1155/2022/5563759

Sun, J.T., Pan, L.L., Zhan, Y., Tsang, D.C.W., Zhu, L.Z. & Li, X.D. (2017). Atrazine contamination in agricultural soils from the Yangtze River Delta of China and associated health risks. *Environmental Geochemistry and Health*, 39(2):369–378. https://doi.org/10.1007/s10653-016-9853-x

Sun, H., Si, C., Bian, Q., Chen, X., Chen, L. & Wang, X. (2012). Developing *in vitro* reporter gene assays to assess the hormone receptor activities of chemicals frequently detected in drinking water. *Journal of Applied Toxicology*, 32(8):635–641. https://doi.org/10.1002/jat.1790

Tabernilla, A., dos Santos Rodrigues, B., Pieters, A., Caufriez, A., Leroy, K., van Campenhout, R., Cooreman, A., Gomes, A.R., Arnesdotter, E., Gijbels, E. & Vinken, M. (2021). *In vitro* liver toxicity testing of chemicals: A pragmatic approach. *International Journal of Molecular Sciences*, 22:5038. https://doi.org/10.3390/ijms22095038

Takeuchi, S., Iida, M., Yabushita, H., Matsuda, T. & Kojima, H. (2008). *In vitro* screening for aryl hydrocarbon receptor agonistic activity in 200 pesticides using a highly sensitive reporter cell line, DR-EcoScreen cells, and *in vivo* mouse liver cytochrome P450-1A induction by propanil, diuron and linuron. *Chemosphere*, 74:155–165. https://doi.org/10.1016/j.chemosphere.2008.08.015

Tan, K., Chen, W., Dong, S., Liu, X., Wang, Y. & Nieh, J. C. (2014). Imidacloprid alters foraging and decreases bee avoidance of predators. *PLoS ONE*, 9(7):e102725. https://doi.org/10.1371/journal.pone.0102725

Tan, Z., Zhou, J., Chen, H., Zou, Q., Weng, S., Luo, T. & Tang, Y. (2016). Toxic effects of 2,4-dichlorophenoxyacetic acid on human sperm function *in vitro*. *Journal of Toxicological Sciences*, 41(4):543–549. https://doi.org/10.2131/jts.41.543

Tang, F.H.M. & Maggie, F. (2021). Pesticide mixtures in soil: a global outlook. *Environmental Research Letters*, 16:044051. https://doi.org/https://doi.org/10.1088/1748-9326/abe5d6

Taxvig, C., Hadrup, N., Boberg, J., Axelstad, M., Bossi, R., Bonefeld-Jørgensen, E.C. & Vinggaard, A.M. (2013). *In vitro - in vivo* correlations for endocrine activity of a mixture of currently used pesticides. *Toxicology and Applied Pharmacology*, 272:757–766. https://doi.org/10.1016/j.taap.2013.07.028

Thongprakaisang, S., Thiantanawat, A., Rangkadilok, N., Suriyo, T. & Satayavivad, J. (2013). Glyphosate induces human breast cancer cells growth via estrogen receptors. *Food and Chemical Toxicology*, 59:129–136. https://doi.org/10.1016/j.fct.2013.05.057

Tian, J., Feng, Y., Fu, H., Xie, H. Q., Jiang, J.X. & Zhao, B. (2015). The aryl hydrocarbon receptor: a key bridging molecule of external and internal chemical signals. *Environmental Science and Technology*, 49:9518–9531. https://doi.org/10.1021/acs.est.5b00385

Tian, X., Hong, X., Yan, S., Li, X., Wu, H., Lin, A. & Yang, W. (2020). Neonicotinoids caused oxidative stress and DNA damage in juvenile Chinese rare minnows (*Gobiocypris rarus*). *Ecotoxicology and Environmental Safety*, 197:110566. https://doi.org/10.1016/j.ecoenv.2020.110566

Timmermans, S., Souffriau, J. & Libert, C. (2019). A general introduction to glucocorticoid biology. *Frontiers in Immunology*, 10:1545. https://doi.org/10.3389/fimmu.2019.01545

Toledo-Ibarra, G.A., Girón-Pérez, M.I., Covantes-Rosales, C.E., Ventura-Ramón, G.H., Pérez-Sánchez, G., López-Torres, A., Diaz-Resendiz, K.J.G., Becerril-Villanueva, E., & Pavnó, L. (2021). Alterations in the non-

neuronal cholinergic system induced by *in-vitro* exposure to diazoxon in spleen mononuclear cells of Nile tilapia (*O. niloticus*). *Fish and Shellfish Immunology*, 108:134–141. https://doi.org/10.1016/j.fsi.2020.11.033

Topal, A., Alak, G., Ozkaraca, M., Yeltekin, A.C., Comaklı, S., Acıl, G., Kokturk, M. & Atamanalp, M. (2017). Neurotoxic responses in brain tissues of rainbow trout exposed to imidacloprid pesticide: Assessment of 8-hydroxy-2-deoxyguanosine activity, oxidative stress and acetylcholinesterase activity. *Chemosphere*, 175:186–191. https://doi.org/10.1016/j.chemosphere.2017.02.047

Tsvetkov, N., Samson-Robert, O., Sood, K., Patel, H.S., Malena, D.A., Gajiwala, P.H., Maciukiewicz, P., Fournier, V. & Zayed, A. (2017). Chronic exposure to neonicotinoids reduces honey bee health near corn crops. *Science*, 356(6345):1395–1397. https://doi.org/10.1126/science.aam7470

Tu, H.T., Silvestre, F., Meulder, B. De, Thome, J.P., Phuong, N.T. & Kestemont, P. (2012). Combined effects of deltamethrin, temperature and salinity on oxidative stress biomarkers and acetylcholinesterase activity in the black tiger shrimp (*Penaeus monodon*). *Chemosphere*, 86:83–91. https://doi.org/10.1016/j.chemosphere.2011.09.022

United States Environmental Protection Agency (US EPA). (2001). Methods for collection, storage and manipulation of sediment for chemical and toxicological analyses: Technical Manual. EPA-823-B-01-002. US. Environmental Protection Agency, Office of Water, Washington, DC.

United States Environmental Protection Agency (US EPA). (2022). What is the endocrine system? https://www.epa.gov/endocrine-disruption/what-endocrine-system Date of access: 4 August 2022.

United States National Research Council (US NRC). (2003). Bioavailability of contaminants in soils and sediments: processes, tools, and applications. Washington, D.C., United States of America: The National Academies Press.

Urbatzka, R., van Cauwenberge, A., Maggioni, S., Viganò, L., Mandich, A., Benfenati, E., Lutz, I. & Kloas, W. (2007). Androgenic and antiandrogenic activities in water and sediment samples from the river Lambro, Italy, detected by yeast androgen screen and chemical analyses. *Chemosphere*, 67:1080–1087. https://doi.org/10.1016/j.chemosphere.2006.11.041

Van Maanen, J., de Vaan, M., Veldstra, B. & Hendrix, W. (2001). Pesticides and nitrate in groundwater and rainwater in the province of Limburg, The Netherlands. *Environmental Monitoring and Assessment*, 72:95–114.

Van Rensburg, L.D., de Clercq, W.P., Barnard, J.H. & du Preez, C.C. (2011). Salinity guidelines for irrigation: Case studies from Water Research Commission projects along the Lower Vaal, Riet, Berg and Breede Rivers. *Water SA*, 37(5):739–750). https://doi.org/10.4314/wsa.v37i5.11

Van Vuuren, L. (2010). Vaalharts - A garden in the desert. *The Water Wheel*, Jan/Feb:21–24.

Van Zijl, M.C., Aneck-Hahn, N.H., Swart, P., Hayward, S., Genthe, B. & de Jager, C. (2017). Estrogenic activity, chemical levels and health risk assessment of municipal distribution point water from Pretoria and Cape Town, South Africa. *Chemosphere*, 186:305–313. https://doi.org/10.1016/j.chemosphere.2017.07.130

Ventura, C., Venturino, A., Miret, N., Randi, A., Rivera, E., Núñez, M. & Cocca, C. (2015). Chlorpyrifos inhibits cell proliferation through ERK1/2 phosphorylation in breast cancer cell lines. *Chemosphere*, 120:343–350. https://doi.org/10.1016/j.chemosphere.2014.07.088

Vermeulen, D. & Usher, B. (2009). Operation and monitoring guidelines and the development of a screening tool for irrigating with coal mine water in Mpumalanga Province, South Africa. *Water SA*, 35(4):379–386. https://doi.org/10.4314/wsa.v35i4.76796

Verwey, P. M. J. & Vermeulen, P. D. (2011). Influence of irrigation on the level, salinity and flow of groundwater at Vaalharts Irrigation Scheme. *Water SA*, 37(2), 115–164. https://doi.org/10.4314/wsa.v37i2.65861

Villeneuve, D.L., Richter, C.A., Blankenship, A.L. & Giesy, J.P. (1999). Rainbow trout cell bioassay-derived relative potencies for halogenated aromatic hydrocarbons: Comparison and sensitivity analysis. *Environmental Toxicology and Chemistry*, 18(5):879–888. https://doi.org/10.1002/etc.5620180510

Vonberg, D., Hofmann, D., Vanderborght, J., Lelickens, A., Köppchen, S., Pütz, T., Burauel, P. & Vereecken, H. (2014). Atrazine soil core residue analysis from an agricultural field 21 years after its ban. *Journal of Environmental Quality*, 43(4):1450–1459. https://doi.org/10.2134/jeq2013.12.0497

Voos, G., Groffmaiv, P. & Pfeil, M. (1994). Laboratory analysis of 2,4-D and dicamba residues in soil. *Journal of Agricultural and Food Chemistry*, 42:2502–2507. https://doi.org/10.1021/jf00047a025

Wang, H. & Joseph, J.A. (1999). Quantifying cellular oxidative stress by dichlorofluorescein assay using microplate reader. *Free Radical Biology and Medicine*, 27(5–6):612–616. https://doi.org/10.1016/S0891-5849(99)00107-0

Wang, X. & Liu, Q. (2020). Spatial and temporal distribution characteristics of triazine herbicides in typical agricultural regions of Liaoning, China. *Bulletin of Environmental Contamination and Toxicology*, 105(6):899–905. https://doi.org/10.1007/s00128-020-03049-8

Weideman, E.A., Perold, V. & Ryan, P.G. (2020). Limited long-distance transport of plastic pollution by the Orange-Vaal River system, South Africa. *Science of The Total Environment*, 727:138653. https://doi.org/10.1016/j.scitotenv.2020.138653

Weis, G.C.C., Assmann, C.E., Mostardeiro, V.B., de Oliveira Alves, A., da Rosa, J.R., Pillat, M.M., de Andrade, C.M., Schetinger, M.R.C., Morsch, V.M.M., da Cruz, I.B.M. & Costabeber, I.H. (2021). Chlorpyrifos pesticide promotes oxidative stress and increases inflammatory states in BV-2 microglial cells: A role in neuroinflammation. *Chemosphere*, 278:130417. https://doi.org/10.1016/j.chemosphere.2021.130417

Weiss, J.M., Hamers, T., Thomas, K.V., van der Linden, S., Leonards, P.E.G. & Lamoree, M.H. (2009). Masking effect of anti-androgens on androgenic activity in European river sediment unveiled by effect-directed analysis. *Analytical and Bioanalytical Chemistry*, 394(5):1385–1397. https://doi.org/10.1007/s00216-009-2807-8

Weizel, A., Schlüsener, M.P., Dierkes, G. & Ternes, T.A. (2018). Occurrence of glucocorticoids, mineralocorticoids, and progestogens in various treated wastewater, rivers, and streams. *Environmental Science and Technology*, 52(9):5296–5307. https://doi.org/10.1021/acs.est.7b06147

Wepener, V., van Dyk, C., Bervoets, L., O'Brien, G., Covaci, A. & Cloete, Y. (2011). An assessment of the influence of multiple stressors on the Vaal River, South Africa. *Physics and Chemistry of the Earth, Parts A/B/C*, 36(14–15): 949–962. https://doi.org/10.1016/j.pce.2011.07.075

Wernersson, A.-S., Carere, M., Maggi, C., Tusil, P., Soldan, P., James, A., Sanchez, W., Dulio, V., Broeg, K., Reifferscheid, G., Buchinger, S., Maas, H., Van Der Grinten, E., O'Toole, S., Ausili, A., Manfra, L., Marziali, L., Polesello, S., Lacchetti, I., Mancini, L., Lilja, K., Linderoth, M., Lundeberg, T., Fjällborg, B., Porsbring, T., Larsson, D.G.J., Bengtsson-Palme, J., Förlin, L., Kienle, C., Kunz, P., Vermeirssen, E., Werner, I., Robinson, C.D., Lyons, B., Katsiadaki, I., Whalley, C., den Haan, K., Messiaen, M., Clayton, H., Lettieri, T., Carvalho, R.N., Gawlik, B.M., Hollert, H., Di Paolo, C., Brack, W., Kammann, U. & Kase, R. (2015). The European technical report on aquatic effect-based monitoring tools under the water framework directive. *Environmental Sciences Europe*, 27:7. https://doi.org/10.1186/s12302-015-0039-4

Węsierska-Gądek, J., Schreiner, T., Maurer, M., Waringer, A. & Ranftler, C. (2007). Phenol red in the culture medium strongly affects the susceptibility of human MCF-7 cells to roscovitine. *Cellular and Molecular Biology Letters*, 12:280–293. https://doi.org/10.2478/s11658-007-0002-5

Wessler, I. & Kirkpatrick, C.J. (2008). Acetylcholine beyond neurons: the non-neuronal cholinergic system in humans. *British Journal of Pharmacology*, 154:1558–1571. https://doi.org/10.1038/bjp.2008.185

Wessler, I., Kilbinger, H., Bittinger, F. & Kirkpatrick, C.J. (2001). The biological role of non-neuronal acetylcholine in plants and humans. *Japanese Journal of Pharmacology*, 85:2–10. https://doi.org/10.1254/jjp.85.2

Wessler, I., Kirkpatrick, C.J. & Racké, K. (1998). Non-neuronal acetylcholine, a locally acting molecule, widely distributed in biological systems: Expression and function in humans. *Pharmacology and Therapeutics*, 77(1):59–79. https://doi.org/10.1016/S0163-7258(97)00085-5

Wessler, I., Kirkpatrick, C.J. & Racké, K. (1999). The cholinergic "pitfall": Acetylcholine, a universal cell molecule in biological systems, including humans. *Clinical and Experimental Pharmacology and Physiology*, 26:198–205. https://doi.org/10.1046/j.1440-1681.1999.03016.x

Wilson, R.D., Geronimo, J. & Armbruster, J. A. (1997). 2,4-D dissipation in field soils after applications of 2,4-D dimethylamine salt and 2,4-D 2-ethylhexyl ester. *Environmental Toxicology and Chemistry*, 16(6):1239–1246. https://doi.org/10.1002/etc.5620160620

Westlund, P. & Yargeau, V. (2017). Investigation of the presence and endocrine activities of pesticides found in wastewater effluent using yeast-based bioassays. *Science of the Total Environment*, 607–608:744–751. https://doi.org/10.1016/j.scitotenv.2017.07.032

Wilson, V.S., Blystone, C.R., Hotchkiss, A.K., Rider, C.V. & Gray, L.E. (2008). Diverse mechanisms of anti-androgen action: Impact on male rat reproductive tract development. *International Journal of Andrology*, 31(2):178–187. https://doi.org/10.1111/j.1365-2605.2007.00861.x

Wilson, V.S., Bobseine, K. & Gray, L.E. (2004). Development and characterization of a cell line that stably expresses an estrogen-responsive luciferase reporter for the detection of estrogen receptor agonist and antagonists. *Toxicological Sciences*, 81:69–77. https://doi.org/10.1093/toxsci/kfh180

Wilson, V.S., Bobseine, K., Lambright, C.R., & Gray, L.E. (2002). A novel cell line, MDA-kb2, that stably expresses an androgen- and glucocorticoid-responsive reporter for the detection of hormone receptor agonists and antagonists. *Toxicological Sciences*, 66:69–81. https://doi.org/10.1093/toxsci/66.1.69

Wirbisky, S.E., Weber, G.J., Sepúlveda, M.S., Lin, T.L., Jannasch, A.S. & Freeman, J.L. (2016). An embryonic atrazine exposure results in reproductive dysfunction in adult zebrafish and morphological alterations in their offspring. *Scientific Reports*, 6:1–13. https://doi.org/10.1038/srep21337

Wu, T., Schoenau, J.J., Li, F., Qian, P., Malhi, S.S., Shi, Y. & Xu, F. (2004). Influence of cultivation and fertilization on total organic carbon and carbon fractions in soils from the Loess Plateau of China. *Soil and Tillage Research*, 77:59–68. https://doi.org/10.1016/j.still.2003.10.002

Wu, H., Zhou, M., Xu, J., Wang, J., Tong, J., Sun, N. & Qian, M. (2022). Determining a wide range of antibiotics and pesticides in poultry feathers using selective accelerated solvent extraction-liquid chromatography-mass spectrometry. *Analytical Methods*, 14(3):250–258. https://doi.org/10.1039/d1ay01764k

Xia, Z.Y., Zhang, L., Zhao, Y., Yan, X., Li, S.P., Gu, T. & Jiang, J.D. (2017). Biodegradation of the herbicide 2,4-dichlorophenoxyacetic acid by a new isolated strain of *Achromobacter* sp. LZ35. *Current Microbiology*, 74(2):193–202. https://doi.org/10.1007/s00284-016-1173-y

Xiao, R., Wang, Z., Wang, C. & Yu, G. (2006). Soil screening for identifying ecological risk stressors using a battery of *in vitro* cell bioassays. *Chemosphere*, 64:71–78. https://doi.org/10.1016/j.chemosphere.2005.11.048

Xu, L.-C., Liu, L., Ren, X.-M., Zhang, M.-R., Cong, N., Xu, A.-Q. & Shao, J.-H. (2008). Evaluation of androgen receptor transcriptional activities of some pesticides *in vitro*. *Toxicology*, 243:59–65. https://doi.org/10.1016/j.tox.2007.09.028

Yan, J., Manelski, R., Vasilas, B. & Jin, Y. (2018). Mobile colloidal organic carbon: An underestimated carbon pool in global carbon cycles? *Frontiers in Environmental Science*, 6:148. https://doi.org/10.3389/fenvs.2018.00148

Yan, S., Meng, Z., Tian, S., Teng, M., Yan, J., Jia, M., Li, R., Zhou, Z. & Zhu, W. (2020). Neonicotinoid insecticides exposure cause amino acid metabolism disorders, lipid accumulation and oxidative stress in ICR mice. *Chemosphere*, 246:125661. https://doi.org/10.1016/j.chemosphere.2019.125661

Yang, C.Z., Yaniger, S.I., Jordan, V.C., Klein, D.J. & Bittner, G.D. (2011). Most plastic products release estrogenic chemicals: A potential health problem that can be solved. *Environmental Health Perspectives*, 119(7):989–996. https://doi.org/10.1289/ehp.1003220

Yao, Q., Lin, M., Wang, Y., Lai, Y., Hu, J., Fu, T., Wang, L.U., Lin, S., Chen, L. & Guo, Y. (2015). Curcumin induces the apoptosis of A549 cells via oxidative stress and MAPK signaling pathways. *International Journal of Molecular Medicine*, 36:1118–1126. https://doi.org/10.3892/ijmm.2015.2327

Yazdan, M. Md. S., Ahad, M.T., Mallick, Z., Mallick, S.P., Jahan, I. & Mazumder, M. (2021). An overview of the glucocorticoids' pathways in the environment and their removal using conventional wastewater treatment systems. *Pollutants*, 1:141–155. https://doi.org/10.3390/pollutants1030012

Yu, S., Kim, T., Yoo, K.H. & Kang, K. (2017). The T47D cell line is an ideal experimental model to elucidate the progesterone-specific effects of a luminal A subtype of breast cancer. *Biochemical and Biophysical Research Communications*, 486:752–758. https://doi.org/10.1016/j.bbrc.2017.03.114

Yuan, X., Shen, J., Zhang, X., Tu, W., Fu, Z. & Jin, Y. (2020). Imidacloprid disrupts the endocrine system by interacting with androgen receptor in male mice. *Science of the Total Environment*, 708:135163. https://doi.org/10.1016/j.scitotenv.2019.135163

Yue, Y., Madsen, S., Hedemann, M.S., Knudsen, K.E.B., Sparsø, F.V., Laursen, A., Jensen, H.M., Knudsen, T.A. & Purup, S. (2019). Effect of food ingredients on glucagon-like peptide-1 secretion in STC-1 and HuTu-80 cells. *International Journal of Food Science and Technology*, 54:3149–3155. https://doi.org/10.1111/ijfs.14247

Želježić, D., Mladinić, M., Žunec, S., Lucić Vrdoljak, A., Kašuba, V., Tariba, B., Živković, T., Marjanović, A. M., Pavičić, I., Milić, M., Rozgaj, R. & Kopjar, N. (2016). Cytotoxic, genotoxic and biochemical markers of insecticide toxicity evaluated in human peripheral blood lymphocytes and an HepG2 cell line. *Food and Chemical Toxicology*, 96:90–106. https://doi.org/10.1016/j.fct.2016.07.036

Zhang, A., Chen, Z., Ahrens, L., Liu, W. & Li, Y.-F. (2012). Concentrations of DDTs and enantiomeric fractions of chiral DDTs in agricultural soils from Zhejiang province, China, and correlations with total organic carbon and pH. *Journal of Agricultural and Food Chemistry*, 60(34):8294–8301. https://doi.org/10.1021/jf3024547

Zhang, C., Schilirò, T., Gea, M., Bianchi, S., Spinello, A., Magistrato, A., Gilardi, G. & di Nardo, G. (2020). Molecular basis for endocrine disruption by pesticides targeting aromatase and estrogen receptor. *International Journal of Environmental Research and Public Health*, 17:5664. https://doi.org/10.3390/ijerph17165664

Zhang, J., Liu, R., Niu, L., Zhu, S., Zhang, Q., Zhao, M., Liu, W. & Liu, J. (2018). Determination of endocrine-disrupting potencies of agricultural soils in China via a battery of steroid receptor bioassays. *Environmental Pollution*, 234:846–854. https://doi.org/10.1016/j.envpol.2017.12.004

Zhang, J.N., Yang, L., Zhang, M., Liu, Y.S., Zhao, J.L., He, L.Y., Zhang, Q.Q. & Ying, G.G. (2019). Persistence of androgens, progestogens, and glucocorticoids during commercial animal manure composting process. *Science of the Total Environment*, 665:91–99. https://doi.org/10.1016/j.scitotenv.2019.02.120

Zhang, Y., Dong, S., Wang, H., Tao, S. & Kiyama, R. (2016). Biological impact of environmental polycyclic aromatic hydrocarbons (ePAHs) as endocrine disruptors. *Environmental Pollution*, 213:809–824. https://doi.org/10.1016/j.envpol.2016.03.050

Zimmermann, M., Grösgen, S., Westwell, M.S. & Greenfield, S.A. (2008). Selective enhancement of the activity of C-terminally truncated, but not intact, acetylcholinesterase. *Journal of Neurochemistry*, 104(1):221–232. https://doi.org/10.1111/j.1471-4159.2007.05045.x

Žunec, S., Kašuba, V., Pavičić, I., Marjanović, A. M., Tariba, B., Milić, M., Kopjar, N., Pizent, A., Vrdoljak, A. L., Rozgaj, R. & Želježić, D. (2016). Assessment of oxidative stress responses and the cytotoxic and genotoxic potential of the herbicide tembotrione in HepG2 cells. *Food and Chemical Toxicology*, 94:64–74. https://doi.org/10.1016/j.fct.2016.05.019

Zvarevashe, W., Krishnannair, S. & Sivakumar, V. (2018). Analysis of austral summer and winter rainfall variability in South Africa using ensemble empirical mode decomposition. *IFAC-PapersOnLine*, 51(5):132–137. https://doi.org/10.1016/j.ifacol.2018.06.223

ANNEXURE A: SUPPLEMENTARY INFORMATION FOR CHAPTER 3.

Table S1 Description of sampling locations in the Mpumalanga province and Vaalharts Valley, Northern Cape province of South Africa.

Nr	Sampling location name	Abbreviation	Coordinates	Description	
			Mpumalanga province sampling locations		
1	Maize field 1	M1	25°46'41.3"S 29°38'31.2"E	The site is located in the vicinity of Middelburg Dam and the Klein Olifants River; it is characterised by flat topography; maize crops were planted (vegetative growth stage: V3*); no irrigation pivots; no weeds between crops; no cattle present; the site is surrounded by other maize fields; and two small farm dams are located in the vicinity of the site (approximately 1 to 2 km).	
2	Maize field 2	M2	25°50'37.3"S 29°39'48.3"E	The site is located in the vicinity of the Klein Olifants River and is characterised by flat topography; maize crops were planted (vegetative growth stage: V5*); no irrigation pivots; no weeds between crops; no cattle present; site is surrounded by other maize fields; and electricity transmission towers are located next to the site.	

Nr	Sampling location name	Abbreviation	Coordinates	Description	
3	Maize field 3	M3	25°53'17.8"S 29°35'02.0"E	The site is located in the vicinity of the Klein Olifants River and is characterised by flat topography; maize crops were planted (vegetative growth stage: V3*); no irrigation pivots; no weeds between crops; no cattle present; site is surrounded by other maize fields; and one small farm dam is located in the vicinity of the site (approximately 1 km).	
4	Maize field 4	M4	26°28'24.8"S 29°49'37.4"E	The site is located in the vicinity of the Life-Water River and is characterised by flat topography; maize crops were planted (vegetative growth stage: V5*); no irrigation pivots; no weeds between crops; no cattle present; and the site is surrounded by other maize fields.	

Nr	Sampling location name	Abbreviation	Coordinates	Description	
5	Maize field 5	M5	26°28'55.5"S 29°44'51.0"E	The site is located in the vicinity of the Life Water River and is characterised by flat topography; maize crops were planted (vegetative growth stage: V2*); no irrigation pivots; no weeds between crops; no cattle present; site is surrounded by other maize fields; and electricity transmission towers are located next to the site.	
6	Maize field 6	M6	26°41'24.2"S 29°47'21.6"E	The site is located in the vicinity of the Life Water River and is characterised by flat topography; maize crops were planted (vegetative growth stage: V4*); no irrigation pivots; weeds present between crops; no cattle present; and the site is surrounded by other maize fields.	

Nr	Sampling location name	Abbreviation	Coordinates	Description	
7	Maize field 7	M7	26°49'22.7"S 29°46'21.9"E	The site is located in the vicinity of the Vaal River and is characterised by flat topography; soybean crops were planted (vegetative growth stage: V1*); no irrigation pivots; no weeds between crops; no cattle present; and the site is surrounded by open cropland and grass fields.	
8	Maize field 8	M8	26°49'43.8"S 29°43'50.2"E	The site is located in the vicinity of the Vaal River and is characterised by flat topography; maize crops were planted (vegetative growth stage: V8*); no irrigation pivots; no weeds between crops; no cattle present; and the site is surrounded by other maize fields.	

Nr	Sampling location name	Abbreviation	Coordinates	Description	
9	Maize Field 9	M9	26°53'05.6"S 29°39'00.7"E	The site is located in the vicinity of the Vaal River and is characterised by flat topography; no crops visible; no irrigation pivots; no weeds between crops; no cattle present; and the site is surrounded by other maize fields with visible irrigation pivots in the distance (approximately 1 km).	
10	Maize field 10	M10	26°53'19.3"S 29°23'54.1"E	The site is located in the vicinity of Grootdraai Dam and the Vaal River; it is characterised by flat topography; no crops visible; no irrigation pivots; no weeds between crops; no cattle present; and the site is surrounded by open grass fields with grazing cattle.	

Nr	Sampling location name	Abbreviation	Coordinates	Description	
11	Maize field 11	M11	26°26'56.0"S 29°47'51.1"E	The site is located next to the Life Water River and is characterised by flat topography; maize crops were planted (vegetative growth stage: V3*); no irrigation pivots; weeds present between crops; grazing cattle present; and the site is surrounded by other maize fields.	
			Vaalharts Valley, Northern Cape province sampling locations		
12	Maize field 12	M12	27°39'40.5"S 24°42'30.7"E	The site is located within the Vaalharts Irrigation Scheme, in the vicinity of the Harts River; it is characterised by flat topography; no crops visible; no irrigation pivots; no weeds present; and the site is surrounded by pecan orchards.	

Nr	Sampling location name	Abbreviation	Coordinates	Description	
13	Maize field 13	M13	27°40'41.8"S 24°42'22.2"E	The site is located within the Vaalharts Irrigation Scheme, in the vicinity of the Harts River; it is characterised by flat topography; winter rotation crops were being harvested; visible irrigation pivots on the field; no weeds were present; and the site is surrounded by pecan orchards.	
14	Maize field 14	M14	27°41'50.3"S 24°42'16.8"E	The site is located within the Vaalharts Irrigation Scheme, in the vicinity of the Harts River; it is characterised by flat topography; winter rotation crops were being harvested; no irrigation pivots present; no weeds were present; site is surrounded by pecan orchards.	

Nr	Sampling location name	Abbreviation	Coordinates	Description	
15	Maize field 15	M15	27°41'44.6"S 24°42'30.3"E	The site is located within the Vaalharts Irrigation Scheme, in the vicinity of the Harts River; it is characterised by flat topography; winter rotation crops were being harvested; no pivots present; no weeds were present; and the site is surrounded by pecan orchards.	
16	Pecan orchard 1	P1	27°42'09.9"S 24°43'53.8"E	The site is located within the Vaalharts Irrigation Scheme, in the vicinity of the Harts River; it is characterised by flat topography; pecan trees are small and not fruit bearing; drip irrigation present; weeds surrounding pecan trees; grass planted between orchard rows; and the site is surrounded by pecan orchards.	

Nr	Sampling location name	Abbreviation	Coordinates	Description	
17	Pecan orchard 2	P2	27°43'16.7"S 24°44'01.8"E	The site is located within the Vaalharts Irrigation Scheme, in the vicinity of the Harts River; it is characterised by flat topography; pecan trees are mature and fruit bearing; drip irrigation present; weeds present between pecan trees; and the site is surrounded by pecan orchards.	
18	Pecan orchard 3	P3	27°43'14.0"S 24°45'51.4"E	The site is located within the Vaalharts Irrigation Scheme, in the vicinity of the Harts River; it is characterised by flat topography; pecan trees are mature and fruit bearing; drip irrigation present; no weeds were present; and the site is surrounded by pecan orchards.	

Nr	Sampling location name	Abbreviation	Coordinates	Description	
19	Pecan orchard 4	P4	27°39'40.5"S 24°42'30.7"E	The site is located within the Vaalharts Irirgation Scheme, in the vicinity of the Harts River; it is characterised by flat topography; pecan trees are mature and fruit bearing; drip irrigation present; no weeds present; and the site is surrounded by maize fields.	

*Vegetative growth stages of maize and soybean describe the vegetative development of the aboveground plant foliage; with Vn indicating the number of collar leaves in maize and trifoliate leaves in soybean.

Table S2 Additional information regarding chemicals, reagents, consumables, and instruments used during this study.

Chemicals and reagents				
CAS number	**Catalogue number**	**Description**	**Brand**	**Supplier**
CAS #71-36-3	#34867	n-Butanol	Honeywell Riedel-de-Haën™, Germany	Sigma-Aldrich (Pty) Ltd, South Africa
CAS #110-54-3	#212-4	n-Hexane	Honeywell Burdick & Jackson®	Sigma-Aldrich (Pty) Ltd, South Africa
CAS #504-17-6	#T5500	2-Thiobarbituric acid	Sigma®	Sigma-Aldrich (Pty) Ltd, South Africa
CAS #1746-01-6	#DRE-GA0901171TO	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	Dr Ehrenstorfer®	Industrial Analytical (Pty) Ltd
CAS #94-75-7	#31518	2,4-Dichlorophenoxyacetic acid	Fluka® Analytical	Sigma-Aldrich (Pty) Ltd, South Africa
CAS #4091-99-0	#D6883	2'-7'Dichlorodihydrofluorescein diacetate	Sigma®	Sigma-Aldrich (Pty) Ltd, South Africa
CAS #50-28-2	#E8875	17β-Oestradiol	Sigma®	Sigma-Aldrich (Pty) Ltd, South Africa
CAS #1866-15-5	#01480	Acetylthiocholine iodide	Sigma®	Sigma-Aldrich (Pty) Ltd, South Africa
CAS #75-05-8	#017-4	Acetonitrile	Honeywell Burdick & Jackson®, Germany	Sigma-Aldrich (Pty) Ltd, South Africa
CAS #51963-61-2	#A30030	Adenosine-5'-triphosphate, disodium salt trihydrate	Melford	Promolab (Pty) Ltd T/A Separations, South Africa
CAS #1066-51-9	#324817	Aminomethylphosphonic	Fluka® Analytical	Sigma-Aldrich (Pty) Ltd, South Africa
CAS #1912-24-9	#45330	Atrazine	Fluka® Analytical	Sigma-Aldrich (Pty) Ltd, South Africa
CAS #1689-84-5	#45356	Bromoxynil	Fluka® Analytical	Sigma-Aldrich (Pty) Ltd, South Africa
CAS #58-08-2	#C0750	Caffeine	ReagentPlus®	Sigma-Aldrich (Pty) Ltd, South Africa
CAS #10043-52-4	#C2661	Calcium chloride	Sigma®	Sigma Aldrich (Pty) Ltd, South Africa
CAS #85-61-0	#C70800	Coenzyme A	Melford	Promolab (Pty) Ltd T/A Separations, South Africa

CAS #52918-63-5	#45423	Deltamethrin	Supelco	Sigma-Aldrich (Pty) Ltd, South Africa
CAS #50-02-2	#D4902	Dexamethasone	Sigma®	Sigma-Aldrich (Pty) Ltd, South Africa
CAS #50-99-7	#G7021	D(+)-glucose	Sigma®	Sigma Aldrich (Pty) Ltd, South Africa
CAS #1918-00-9	#45430	Dicamba	Fluka® Analytical	Sigma Aldrich (Pty) Ltd, South Africa
CAS #67-43-6	#D6518	Diethylenetriaminepentaacetic acid	Sigma®	Sigma-Aldrich (Pty) Ltd, South Africa
CAS #67-68-5	#34869	Dimethyl sulphoxide	Chromosalv® Plus, Honeywell Riedel-de-Haën™, Germany	Sigma-Aldrich (Pty) Ltd, South Africa
CAS #7758-11-4	#1051041000	Dipotassium hydrogen phosphate	Sigma®	Sigma Aldrich (Pty) Ltd, South Africa
CAS #3483-12-3	#D11000	Dithiolthreitol	Melford	Promolab (Pty) Ltd T/A Separations, South Africa
CAS #6381-92-6	#E4884	Ethylenediaminetetraacetic acid-disodium salt	Sigma®	Sigma-Aldrich (Pty) Ltd, South Africa
CAS #69-78-3	#D218200	Ellman’s reagent	Sigma®	Sigma-Aldrich (Pty) Ltd, South Africa
CAS #64-17-5	#1.11727.2500	Ethanol	LiChrosolv®, Supelco	Sigma-Aldrich (Pty) Ltd, South Africa
CAS #13311-84-7	#F9397	Flutamide	Sigma®	Sigma-Aldrich (Pty) Ltd, South Africa
CAS #64-18-6	#56302	Formic acid	Honeywell FlukaTM	Sigma-Aldrich (Pty) Ltd, South Africa
CAS #129453-61-8	#EPY0001399	Fulvestrant	European Pharmacopoeia Reference Standard	Industrial Analytical (Pty) Ltd, South Africa
CAS #64-19-7	#A6283	Glacial acetic acid	Merck	Merck Chemicals (Pty) Ltd, South Africa
CAS #1071-83-6	#45521	Glyphosate	Fluka® Analytical	Sigma-Aldrich (Pty) Ltd, South Africa
CAS #7365-45-9	#25-060-CI	HEPES buffer	Corning®	The Scientific Group (Pty) Ltd, South Africa
CAS #7647-01-0	#1.00319	Hydrochloric acid	Emsure®	Sigma-Aldrich (Pty) Ltd, South Africa

CAS #7722-84-1	#H1009	Hydrogen peroxide	Sigma®	Sigma-Aldrich (Pty) Ltd, South Africa
CAS #138261-41-3	#37894	Imidacloprid	Fluka® Analytical	Sigma-Aldrich (Pty) Ltd, South Africa
CAS #56378-72-4	#M5671	Magnesium carbonate hydroxide pentahydrate	Sigma®	Sigma Aldrich (Pty) Ltd, South Africa
CAS #10034-99-8	#23,039	Magnesium sulphate heptahydrate	Sigma®	Sigma Aldrich (Pty) Ltd, South Africa
CAS #67-56-1	#230-4	Methanol	Honeywell Burdick & Jackson®, Germany	Sigma-Aldrich (Pty) Ltd, South Africa
CAS #298-93-1	#M5655	MTT (-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)	Sigma®	Sigma Aldrich (Pty) Ltd, South Africa
CAS #7778-77-0	#P5655	Potassium dihydrogen phosphate	Sigma®	Sigma-Aldrich (Pty) Ltd, South Africa
CAS #7722-64-7	#223468	Potassium permanganate	Sigma®	Sigma-Aldrich (Pty) Ltd, South Africa
CAS #110-86-1	#P57506	Pyridine	ReagentPlus®	Sigma-Aldrich (Pty) Ltd, South Africa
CAS #87-66-1	#16040	Pyrogallol	Sigma®	Sigma-Aldrich (Pty) Ltd, South Africa
CAS #144-55-8	#S5761	Sodium bicarbonate	Sigma®	Sigma Aldrich (Pty) Ltd, South Africa
CAS #151-21-3	#L4509	Sodium dodecyl sulphate	Sigma®	Sigma Aldrich (Pty) Ltd, South Africa
CAS #57-50-1	#S0389	Sucrose	Sigma®	Sigma-Aldrich (Pty) Ltd, South Africa
CAS #7664-93-9	#SAAR5885124LL	Sulphuric acid	Merck	Merck (Pty) Ltd
CAS #58-22-0	#86500	Testosterone	Fluka® Analytical	Sigma-Aldrich (Pty) Ltd, South Africa
CAS #5704-04-1	#T5816	Tricine	Sigma®	Sigma-Aldrich (Pty) Ltd, South Africa
CAS #1185-53-1	#10812846001	Tris-HCl	Trizma®	Sigma-Aldrich (Pty) Ltd
CAS #72-57-1	#T8154	Trypan blue solution	Sigma®	Sigma-Aldrich (Pty) Ltd, South Africa
Media constituents and reagents				
Catalogue number	**Description**		**Brand**	**Supplier**

#L0010-100	Antibiotic-antimycotic mixture	Biowest	Celtic Molecular Diagnostics (Pty) Ltd, South Africa
#E1601	Beetle luciferin, potassium salt	Promega	Promolab (Pty) Ltd T/A Separations, South Africa
#B6919	Bradford's reagent	Supelco	Sigma Aldrich (Pty) Ltd, South Africa
#C6241	Charcoal, dextran coated	Sigma®	Sigma Aldrich (Pty) Ltd, South Africa
#SH30068.03	Charcoal dextran treated foetal bovine serum	HyCloneTM Cytiva	Promolab (Pty) Ltd T/A Separations, South Africa
#D2902	Dulbecco's Modified Eagle's Medium with low glucose and without phenol red	Sigma®	Sigma Aldrich (Pty) Ltd, South Africa
#D5652	Dulbecco's phosphate-buffered saline	Sigma®	Sigma-Aldrich (Pty) Ltd, South Africa
#FBS-GI-12A	Foetal bovine serum, gamma-irradiated, South American origin	Capricorn Scientific	Biocom Africa (Pty) Ltd, South Africa
#4386	Leibovitz medium containing L-glutamine	Sigma®	Sigma Aldrich (Pty) Ltd, South Africa
#E3971	Reporter lysis buffer	Promega	Anatech Instruments (Pty) Ltd, South Africa
#R8755	Rosswell Park Memorial Institute 1640 medium	Sigma®	Sigma Aldrich (Pty) Ltd, South Africa
#S8636	Sodium pyruvate solution	Sigma®	Sigma-Aldrich (Pty) Ltd, South Africa
#X0930-100	Trypsin-EDTA	Biowest	Celtic Molecular Diagnostics (Pty) Ltd, South Africa
Consumables			
Catalogue number	**Product**	**Brand**	**Supplier**
#596-4520	0.22 µM polyethersulfone bottle-top filter	Nalgene®	AEC-Amersham SOC Ltd
#4187	0.22 µm Syringe filter	Pall Acrodisc®	Promolab (Pty) Ltd T/A Separations, South Africa
#P2TUB003C	2 mL Microcentrifuge tube	Axygen	Lasec (Pty) Ltd, South Africa

#F1016S	3 mL Plastic Pasteur pipette	LabocareTM	SymbioLab (Pty) Ltd, South Africa
#GSP110010	10 mL Serological pipette	Jet Biofil®	Symbiolab (Pty) Ltd, South Africa
#4101	10 mL Serological pipette	StripetteTM	The Scientific Group (Pty) Ltd, South Africa
#652000 001	10 mL Syringe	Surgi Plus	Symbiolab (Pty) Ltd, South Africa
#352096	15 mL Centrifuge tube	Falcon®	Lasec (Pty) Ltd, South Africa
#352096	15 mL Centrifuge tubes	Corning®	The Scientific Group (Pty) Ltd, South Africa
#3527	24-Well, clear, flat bottom cell culture microplates	Corning® Costar®	The Scientific Group (Pty) Ltd, South Africa
#352070	50 mL Centrifuge tube	Falcon®	Lasec SA (Pty) Ltd, South Africa
#93100	60.1 cm2 Tissue culture dish	TPP	Promolab (Pty) Ltd T/A Separations, South Africa
#430168	60.1 cm2 Tissue culture dishes	Corning®	The Scientific Group (Pty) Ltd, South Africa
#3924	96-Well, black, flat bottom cell culture microplate	Corning®,	Sigma-Aldrich (Pty) Ltd, South Africa
#92096	96-Well, clear, flat bottom cell culture test plates	TPP	Promolab Pty Ltd T/A Separations, South Africa
#655098	96-Well, clear flat bottom, white-walled cell culture microplates	Cellstar® Greiner Bio-One	Lasec (Pty) Ltd, South Africa
#L12011	Cell counting slide	LUNATM	Logos Biosystems Inc
#2104-0008	High-density polyethylene bottles	Thermo Scientific™ Nalgene®	AEC-Amersham SOC Ltd, South Africa
#760175	High efficiency particulate air filters	Thermo ScientificTM	Labotec SA (Pty) Ltd, South Africa
#17804	In-line filters	Sartorius, Midisart® 2000	Labotec (Pty) Ltd, South Africa
Equipment			
Model	**Description**	**Brand**	**Supplier**

#05060793	Accelerated Solvent Extractor	Dionex ASE 100®	Thermo Scientific, United Stated of America
#059703	ASE extraction cells	ASE 100®	Thermo Scientific, United Stated of America
#L40002	Automated bright-field cell counter	LUNA-II™	Logos Biosystems Inc
	Biosafety cabinet	BiofFlow-II	Labotec (Pty) Ltd, South Africa
#Z 32 HK	Centrifuge	Hermle Labortechnik GmbH	Lasec (Pty) Ltd, South Africa
#ULTRA GE MK2	ELGA water purification system	PURELAB® Ultra MK2 version 3 11/08	Labotec (Pty) Ltd, South Africa
#Eclipse TS100-F	Inverted phase-contrast microscope	Nikon	Nikon Instruments Inc, United States of America
#SP015+UPF75	Mechanical shaker	Labcon®	Labcon Laboratory Equipment (Pty) Ltd, South Africa
#L51/S R	Muffle furnace	Nabertherm GmbH	Labotec (Pty) Ltd
#49178-21	Multimode microplate reader	TriStar LB 941	Berthold Technologies, Germany
#5054747 H	Multimode microplate reader	SpectraMax® iD3 Molecular Devices, LCC	Lasec SA (Pty) Ltd, South Africa
#138631	Steel mesh sieve	Clear Edge Test Sieves	Clear Edge Test Sieves, South Africa
# P/N 103196/0	Turbovap concentrator	TurboVap® II	Caliper Life Sciences Inc, United States of America
#704	Ultrasonic bath	Scientech	Labotec (Pty) Ltd, South Africa
#WBESPL2S	Water bath	Labcon®	Labdesign Engineering (Pty) Ltd, South Africa

ANNEXURE B: SUPPLEMENTARY INFORMATION FOR CHAPTER 4.

Table S3 *p*-values used to determine statistical significance of cell viability and responses of the different reporter gene bioassays after the respective cell lines were exposed to the samples.

Sample name	Exposure concentration (mg/mL)	H4IIE-*luc* bioassay		MDA-kb2 bioassay		MDA-kb2 bioassay		T47D-KB*luc*		T47D-KB*luc*	
		Cell viability	AhR↑	Cell viability	AR↑	Cell viability	AR↓	Cell viability	ER↑	Cell viability	ER↓
Controls											
BC	N/A	0.009*	0.001*	0.300	0.022*	0.289	0.337	0.354	0.515	0.431	0.813
NC	N/A	0.005*	-	0.011*	-	0.005*	-	0.005*	-	0.005*	-
SC	N/A	-	-	-	-	-	-	-	-	-	-
Mpumalanga province sampling locations											
M1	1	-	0.877	-	0.009*	-	0.009*	-	0.900	-	0.060
	3	-	0.105	-	0.033*	-	0.021*	-	0.150	-	0.091
	9	-	0.165	-	0.012*	-	0.021*	-	0.381	-	0.707
	28	0.184	0.758	0.825	0.020*	0.658	0.005*	0.275	0.381	0.513	0.348
	83	0.275	0.190	0.507	0.452	0.513	0.005*	0.827	0.009*	0.275	0.052
	250	0.827	0.190	0.046*	0.005*	0.827	0.007*	0.275	0.005*	0.827	0.573
M2	1	-	0.877	-	0.009*	-	0.006*	-	0.159	-	0.052
	3	-	0.817	-	0.045*	-	0.028*	-	0.060	-	0.661
	9	-	0.758	-	0.017*	-	0.007*	-	0.069	-	0.416
	28	0.127	0.316	0.825	0.012*	0.050*	0.009*	0.050*	0.900	0.513	0.348
	83	0.275	0.217	0.825	0.452	0.827	0.009*	0.275	0.015*	0.513	0.416
	250	0.050*	*0.007	0.825	0.348	†	†	0.050*	0.005*	0.513	0.015*
M3	1	-	0.280	-	0.707	-	0.028*	-	0.091	-	0.234
	3	-	0.877	-	0.851	-	0.754	-	0.012*	-	0.802
	9	-	1.000	-	0.316	-	0.210	-	0.010*	-	0.210
	28	0.050*	0.939	0.046*	0.316	0.268	0.009*	0.275	0.009*	0.050*	0.616
	83	0.050*	0.487	0.825	0.260	0.658	0.009*	0.127	0.006*	0.827	0.452
	250	0.827	0.643	0.268	0.661	0.050*	0.661	0.050*	0.005*	0.513	0.045*
M4	1	-	0.700	-	0.168	-	0.802	-	0.07*	-	0.287
	3	-	0.939	-	0.103	-	0.452	-	0.010*	-	0.260
	9	-	0.440	-	0.042*	-	0.661	-	0.007*	-	0.802
	28	0.513	0.247	0.825	0.150	0.827	0.851	0.827	0.007*	0.827	0.573
	83	0.827	0.064	0.046*	0.117	0.827	0.381	0.513	0.005*	0.658	0.616
	250	0.827	0.280	0.046*	0.210	0.827	0.017*	0.050*	0.005*	0.827	0.011*
M5	1	-	0.939	-	0.117	-	0.009*	-	0.007*	-	0.080

Sample name	Exposure concentration (mg/mL)	H4IIE-*luc* bioassay		MDA-kb2 bioassay		MDA-kb2 bioassay		T47D-KB*luc*		T47D-KB*luc*	
		Cell viability	AhR↑	Cell viability	AR↑	Cell viability	AR↓	Cell viability	ER↑	Cell viability	ER↓
	3	-	0.700	-	0.168	-	0.033*	-	0.007*	-	0.491
	9	-	0.165	-	0.616	-	0.009*	-	0.005*	-	0.802
	28	0.275	0.190	0.046*	0.416	0.827	0.009*	0.513	0.006*	1.000	0.316
	83	0.513	0.105	0.046*	0.316	0.127	0.005*	0.127	0.005*	0.275	0.851
	250	0.827	0.817	0.046*	0.017*	0.127	0.005*	0.275	0.005*	0.050*	0.133
M6	1	-	0.090	-	0.616	-	0.022*	-	0.009*	-	0.024*
	3	-	0.064	-	0.754	-	0.009*	-	0.012*	-	0.060
	9	-	0.054	-	0.802	-	0.052	-	0.009*	-	0.103
	28	0.513	*0.025	0.827	0.950	0.827	0.007*	0.050*	0.017*	0.275	0.260
	83	0.827	0.143	0.275	0.015*	0.127	0.005*	0.827	0.005*	0.513	0.103
	250	0.513	0.247	0.658	0.005*	0.513	0.009*	0.050*	0.005*	0.275	0.017*
M7	1	-	0.064	-	0.033*	-	0.007*	-	0.017*	-	0.234
	3	-	0.190	-	0.091	-	0.316	-	0.012*	-	0.287
	9	-	0.537	-	0.260	-	0.039*	-	0.012*	-	0.234
	28	0.050*	*0.031	0.376	0.010*	0.046*	0.010*	0.513	0.010*	0.827	0.900
	83	0.050*	*0.017	0.275	0.020*	0.046*	0.007*	0.275	0.009*	0.827	1.000
	250	0.275	*0.005	0.376	0.348	0.127	0.007*	0.513	0.005*	0.275	0.024*
M8	1	-	*0.017	-	0.168	-	0.007*	-	0.012*	-	0.707
	3	-	0.143	-	0.133	-	0.210	-	0.010*	-	0.348
	9	-	0.939	-	0.150	-	0.150	-	0.012*	-	0.381
	28	0.127	*0.021	0.658	0.045*	0.050*	0.052	0.513	0.012*	0.275	0.234
	83	0.050*	0.316	0.658	0.009*	0.513	0.009*	0.275	0.006*	0.827	0.802
	250	0.050*	*0.011	0.827	0.012*	0.127	0.005*	0.827	0.005*	0.127	0.754
M9	1	-	*0.007	-	0.416	-	0.491	-	0.069	-	0.168
	3	-	*0.017	-	0.080	-	0.754	-	0.052	-	0.900
	9	-	0.217	-	0.661	-	0.851	-	0.017*	-	0.707
	28	0.827	0.939	0.121	0.851	0.050*	0.661	0.513	0.015*	0.827	0.900
	83	0.827	0.105	0.077	0.091	0.275	0.452	0.827	0.052	0.827	0.661
	250	0.127	*0.005	0.184	0.452	0.127	0.009*	0.827	0.005*	0.127	0.900
M10	1	-	0.054	-	0.188	-	0.188	-	0.234	-	0.900
	3	-	0.123	-	0.168	-	0.287	-	0.900	-	0.573
	9	-	0.203	-	0.117	-	0.348	-	0.348	-	0.707
	28	0.827	0.643	0.050*	0.009*	0.658	0.950	0.050*	0.012*	0.827	0.851
	83	0.050*	0.316	0.827	0.573	0.050*	0.210	0.827	0.006*	0.827	0.851
	250	0.050*	0.537	0.827	0.024*	0.275	0.021*	0.275	0.005*	0.513	0.039*

Sample name	Exposure concentration (mg/mL)	H4IIE-*luc* bioassay		MDA-kb2 bioassay		MDA-kb2 bioassay		T47D-KB*luc*		T47D-KB*luc*	
		Cell viability	AhR↑	Cell viability	AR↑	Cell viability	AR↓	Cell viability	ER↑	Cell viability	ER↓
M11	1	-	0.165	-	0.234	-	0.531	-	0.348	-	0.316
	3	-	0.143	-	0.316	-	0.210	-	0.287	-	0.452
	9	-	0.190	-	0.080	-	0.287	-	0.348	-	0.348
	28	0.513	0.045*	0.077	0.033*	0.050*	0.531	0.513	0.900	0.127	0.531
	83	0.513	0.025*	0.050*	0.381	0.050*	0.117	0.513	0.754	0.827	0.491
	250	0.827	0.877	0.513	0.005*	0.127	0.039*	0.827	0.012*	0.275	0.210
Vaalharts Valley, Northern Cape province sampling locations											
M12	1	-	0.817	-	0.900	-	0.103	-	0.039*	-	0.416
	3	-	0.700	-	0.851	-	0.103	-	0.024*	-	0.950
	9	-	0.440	-	0.348	-	0.069	-	0.021*	-	0.150
	28	0.658	0.031*	0.376	0.491	0.275	0.060	0.513	0.616	0.513	0.661
	83	0.827	0.054	0.513	0.133	0.050*	0.080	0.513	0.069	0.513	0.416
	250	0.275	0.045*	0.827	0.188	0.050*	0.069	0.275	0.005*	0.050*	0.851
M13	1	-	0.165	-	0.052	-	0.616	-	0.010*	-	0.028*
	3	-	0.090	-	0.009*	-	0.754	-	0.009*	-	0.015*
	9	-	0.064	-	0.009*	-	0.950	-	0.007*	-	0.133
	28	0.827	0.396	0.827	0.039*	0.121	0.802	0.275	0.009*	0.827	0.012*
	83	0.827	0.031*	0.376	0.069	0.513	0.802	0.827	0.009*	0.050*	0.010*
	250	0.127	0.123	0.184	0.150	0.513	0.210	0.827	0.005*	0.275	0.017*
M14	1	-	0.021*	-	0.007*	-	0.060	-	0.010*	-	0.260
	3	-	0.263	-	0.009*	-	0.045*	-	0.010*	-	0.021*
	9	-	0.090	-	0.009*	-	0.091	-	0.010*	-	0.009*
	28	0.827	0.009*	0.077	0.009*	0.513	0.234	0.827	0.069	0.217	0.0210*
	83	0.827	0.021*	0.077	0.348	0.827	0.091	0.513	0.012*	0.217	0.028*
	250	0.275	0.005*	0.046*	0.348	0.050*	0.033*	0.513	0.010*	0.513	0.573
M15	1	-	0.316	-	0.531	-	0.573	-	0.381	-	0.150
	3	-	0.045*	-	0.150	-	0.851	-	0.234	-	0.033*
	9	-	0.031*	-	0.024*	-	0.210	-	0.287	-	0.024*
	28	0.513	0.005*	0.658	0.060	0.513	0.573	0.275	0.348	0.050*	0.103
	83	0.513	0.011*	0.658	0.009*	0.827	0.900	0.513	0.039*	0.127	0.052
	250	0.827	0.877	0.127	0.802	0.513	0.024*	0.513	0.005*	0.050*	0.024*
P1	1	-	0.247	-	0.754	-	0.348	-	0.573	-	0.416
	3	-	0.143	-	0.103	-	0.117	-	0.381	-	0.168
	9	-	0.045*	-	0.491	-	0.707	-	0.234	-	0.150

Sample name	Exposure concentration (mg/mL)	H4IIE-*luc* bioassay		MDA-kb2 bioassay		MDA-kb2 bioassay		T47D-KB*luc*		T47D-KB*luc*	
		Cell viability	AhR↑	Cell viability	AR↑	Cell viability	AR↓	Cell viability	ER↑	Cell viability	ER↓
	28	0.513	0.011*	0.513	0.316	0.127	0.754	0.275	0.452	0.376	0.080
	83	0.827	0.011*	0.275	0.531	0.127	0.234	0.513	0.950	0.827	0.039*
	250	0.050*	0.031*	0.275	0.012*	0.127	0.060	0.827	0.017*	0.513	0.045*
P2	1	-	0.939	-	0.900	-	0.007*	-	0.802	-	0.616
	3	-	0.076	-	0.103	-	0.024*	-	0.009*	-	0.900
	9	-	0.143	-	0.876	-	0.007*	-	0.110	-	0.012*
	28	0.127	0.005*	0.275	0.060	0.050*	0.007*	0.827	0.398	0.513	0.133
	83	0.275	0.076	0.050*	0.316	0.050*	0.006	0.275	0.950	0.127	0.150
	250	0.050*	0.700	0.513	0.012*	†	†	0.827	0.007*	0.827	0.168
P3	1	-	0.076	-	0.416	-	0.012*	-	0.900	-	0.260
	3	-	0.025*	-	0.080	-	0.060	-	0.616	-	1.000
	9	-	0.045*	-	0.168	-	0.009*	-	0.210	-	0.348
	28	0.050*	0.009*	0.050*	0.020*	0.050*	0.010*	0.827	0.080	0.275	0.531
	83	0.658	0.054	0.077	0.754	0.275	0.006*	0.827	0.015*	0.513	0.052
	250	0.275	0.316	0.513	0.005*	0.050*	0.005*	0.827	0.009*	0.827	0.007*
P4	1	-	0.031*	-	0.012*	-	0.117	-	0.005*	-	0.021*
	3	-	0.045*	-	0.015*	-	0.133	-	0.007*	-	0.010*
	9	-	0.025*	-	0.012*	-	0.033*	-	0.017*	-	0.150
	28	0.127	0.011*	0.857	0.012*	0.275	0.348	0.513	0.012*	0.050*	0.080
	83	0.050*	0.025*	0.050*	0.009*	0.827	0.381	0.827	0.009*	0.507	0.150
	250	0.050*	0.939	0.050*	0.052	0.275	0.069	0.513	0.005*	0.127	0.851

↑agonism; ↓antagonism; *statistically significant compared to the solvent control (SC) ($p \leq 0.05$); -concentrations were not investigated; †could not be determined due to the presence of bacteria in the wells of the 96-well cell culture test plate; AhR: aryl hydrocarbon receptor; AR: androgen receptor; BC: blank control (untreated cells); ER: oestrogen receptor; M1–M15: Maize field 1–Maize field 15; N/A: not applicable; NC: negative control (cells treated with methanol, i.e., non-viable cells); P1–P4: Pecan orchard 1–Pecan orchard 4.

Table S4 Results obtained for the H_2DCF-DA bioassay in which the induction of reactive oxygen species production in HuTu-80 cells was assessed after exposure to samples (83 mg/mL) for 24 hours.

Sample name	RFUs	*p*-value	d-value
Controls			
BC	39 691 ± 16 222	-	-
PC	53 617 ± 7 562	0.072	**0.9**
Mpumalanga province sampling locations			
M1	**60 795 ± 8 487	0.006	**1.3**
M2	**74 896 ± 8 265	0.001	**2.2**
M3	**60 658 ± 15 635	0.009	**1.3**
M4	*55 513 ± 8 806	0.012	**1.0**
M5	**79 877 ± 21 732	0.005	**2.5**
M6	**12 862 ± 3 935	0.001	**1.7**
M7	**9 559 ± 1 888	0.001	**1.9**
M8	**11 521 ± 2 203	0.001	**1.7**
M9	**13 457 ± 3 990	0.001	**1.6**
M10	**12 972 ± 4 182	0.001	**1.6**
M11	**12 804 ± 1 102	0.001	**1.7**
Vaalharts Valley, Northern Cape province sampling locations			
M12	35 788 ± 10 495	0.123	0.2
M13	27 671 ± 8 847	0.150	0.7
M14	37 884 ± 16 282	0.440	0.1
M15	44 886 ± 31 143	1.000	0.3
P1	*60 604 ± 13 934	0.027	**1.3**
P2	42 425 ± 16 043	0.681	0.2
P3	26 722 ± 8 677	0.959	**0.8**
P4	36 402 ± 10 305	0.959	0.2

Data is presented as the mean ± standard deviation; asterisks indicate statistically significant differences compared to the blank control (untreated cells) (*$p \leq 0.05$ and **$p \leq 0.01$); values bold indicate practically significant differences compared to the control (untreated cells) ($d \geq 0.8$); BC: blank control; M1–M15: Maize field 1–Maize field 15; P1–P4: Pecan orchard 1–Pecan orchard 4; PC: positive control (i.e., cells stimulated with 3.5 ng/mL hydrogen peroxide for 45 minutes); RFUs: relative fluorescence units.

Table S5 Results obtained for the H_2DCF-DA bioassay in which the induction of reactive oxygen species production in H4IIE-*luc* cells was assessed after exposure to samples (83 mg/mL) for 24 hours.

Sample name	RFUs	*p*-value	d-value
Controls			
BC	37 837 ± 8 303	-	-
PC	**69 628 ± 30 366	0.001	**3.8**
Mpumalanga province sampling locations			
M1	**65 713 ± 21 688	0.002	**3.4**
M2	36 349 ± 5 110	0.841	0.2
M3	42 548 ± 14 101	0.221	0.6
M4	*30 794 ± 4 190	0.036	**0.8**
M5	**54 919 ± 10 508	0.001	**2.1**
M6	38 214 ± 3 816	0.898	0.0
M7	*42 576 ± 4 137	0.036	0.6
M8	31 108 ± 8 322	0.812	**0.8**
M9	30 415 ± 3 093	0.985	**0.9**
M10	43 517 ± 5 361	0.956	0.7
M11	33 301 ± 7 487	0.454	0.5
Vaalharts Valley, Northern Cape province sampling locations			
M12	**67 580 ± 18 337	0.001	**3.6**
M13	*57 962 ± 20 845	0.014	**2.4**
M14	**60 888 ± 14 667	0.001	**2.8**
M15	**27 261 ± 2 754	0.002	**1.3**
P1	33 741 ± 9 170	0.333	0.5
P2	44 134 ± 15 070	0.596	**0.8**
P3	**17 888 ± 3 178	0.001	**2.4**
P4	**19 379 ± 4 730	0.001	**2.2**

Data is presented as the mean ± standard deviation; asterisks indicate statistically significant differences compared to the blank control (untreated cells) (*$p \leq 0.05$ and **$p \leq 0.01$); values in bold indicate practically significant differences compared to the control (untreated cells) ($d \geq 0.8$); BC: blank control; M1–M15: Maize field 1–Maize field 15; P1–P4: Pecan orchard 1–Pecan orchard 4; PC: positive control (i.e., cells stimulated with 3.5 ng/mL hydrogen peroxide for 45 minutes); RFUs: relative fluorescence units.

Table S6 Superoxide dismutase content in HuTu-80 cells after exposure to samples (83 mg/mL) for 24 hours.

Sample name	SOD content (ng SOD/mg protein)	*p*-value	d-value
Control			
BC	122.74 ± 62.33	-	-
Mpumalanga province sampling locations			
M1	*160.23 ± 11.20	0.025	0.6
M2	159.22 ± 43.57	0.121	0.6
M3	*162.74 ± 22.13	0.036	0.6
M4	161.98 ± 44.39	0.107	0.6
M5	142.08 ± 36.85	0.432	0.3
M6	154.44 ± 12.12	0.084	0.5
M7	*182.14 ± 35.70	0.013	**1.0**
M8	**10.72 ± 8.36	0.001	**1.8**
M9	126.72 ± 34.66	0.956	0.1
M10	*180.04 ± 61.49	0.036	**0.9**
M11	**289.33 ± 79.09	0.001	**2.7**
Vaalharts Valley, Northern Cape province sampling locations			
M12	*62.45 ± 63.62	0.023	**1.0**
M13	**11.18 ± 7.47	0.001	**1.8**
M14	**48.53 ± 72.49	0.008	**1.2**
M15	**44.87 ± 69.86	0.001	**1.2**
P1	**21.31 ± 15.99	0.001	**1.6**
P2	**21.09 ± 28.68	0.001	**1.6**
P3	**39.60 ± 45.14	0.003	**1.3**
P4	**47.27 ± 21.11	0.009	**1.2**

Data is presented as the mean ± standard deviation; asterisks indicate statistically significant differences compared to the blank control (untreated cells) (*$p \leq 0.05$ and **$p \leq 0.01$); values in bold indicate practically significant differences compared to the control (untreated cells) ($d \geq 0.8$); BC: blank control; M1–M15: Maize field 1–Maize field 15; P1–P4: Pecan orchard 1–Pecan orchard 4; SOD: superoxide dismutase.

Table S7 Superoxide dismutase content in H4IIE-*luc* cells after exposure to samples (83 mg/mL) for 24 hours.

Sample name	SOD content (ng SOD/mg protein)	*p*-value	d-value
Control			
BC	68.97 ± 35.91	-	-
Mpumalanga province sampling locations			
M1	72.70 ± 32.76	0.615	0.1
M2	*110.52 ± 66.62	0.039	**1.2**
M3	93.74 ± 39.58	0.089	0.7
M4	98.85 ± 43.63	0.058	**0.8**
M5	**113.75 ± 54.41	0.010	**1.2**
M6	**118.94 ± 61.19	0.008	**1.4**
M7	**135.91 ± 82.86	0.009	**1.9**
M8	**106.68 ± 34.24	0.007	**1.1**
M9	91.05 ± 37.53	0.067	0.6
M10	59.46 ± 34.26	0.419	0.3
M11	*40.29 ± 37.46	0.015	**0.8**
Vaalharts Valley, Northern Cape province sampling locations			
M12	78.91 ± 33.12	0.419	0.3
M13	84.32 ± 44.59	0.332	0.4
M14	78.08 ± 26.70	0.362	0.3
M15	57.44 ± 37.83	0.383	0.3
P1	71.78 ± 25.76	0.689	0.1
P2	54.02 ± 28.33	0.260	0.4
P3	66.85 ± 24.63	0.899	0.1
P4	57.43 ± 42.72	0.326	0.3

Data is presented as the mean ± standard deviation; asterisks indicate statistically significant differences compared to the blank control (untreated cells) (*$p \leq 0.05$ and **$p \leq 0.01$); values in bold indicate practically significant differences compared to the control (untreated cells) ($d \geq 0.8$); BC: blank control; M1–M15: Maize field 1–Maize field 15; P1–P4: Pecan orchard 1–Pecan orchard 4; SOD: superoxide dismutase.

Table S8 Catalase activity in HuTu-80 cells after exposure to samples (83 mg/mL) for 24 hours.

Sample name	CAT activity (µM H_2O_2/min/mg protein)	*p*-value	d-value
Control			
BC	27.87 ± 21.79	-	-
Mpumalanga province sampling locations			
M1	17.23 ± 3.74	0.841	0.5
M2	12.43 ± 2.55	0.051	0.7
M3	14.98 ± 3.24	0.547	0.6
M4	15.16 ± 3.30	0.609	0.6
M5	13.31 ± 3.41	0.107	0.7
M6	12.83 ± 3.05	0.120	0.7
M7	48.60 ± 19.50	0.059	**1.0**
M8	**60.60 ± 20.98	0.001	**1.5**
M9	*12.13 ± 3.57	0.036	0.7
M10	*44.38 ± 15.97	0.022	**0.8**
M11	**62.17 ± 22.33	0.001	**1.6**
Vaalharts Valley, Northern Cape province sampling locations			
M12	**56.13 ± 13.82	0.004	**1.3**
M13	*43.74 ± 7.86	0.036	0.7
M14	**54.18 ± 15.14	0.006	**1.2**
M15	*46.25 ± 21.95	0.020	**0.8**
P1	32.92 ± 12.95	0.298	0.2
P2	*47.17 ± 17.99	0.028	**0.9**
P3	*50.32 ± 27.90	0.035	**1.0**
P4	*46.74 ± 14.22	0.028	**0.9**

Data is presented as the mean ± standard deviation; asterisks indicate statistically significant differences compared to the blank control (untreated cells) (*$p \leq 0.05$ and **$p \leq 0.01$); values in bold indicate practically significant differences compared to the control (untreated cells) ($d \geq 0.8$); BC: blank control; CAT: catalase; H_2O_2: hydrogen peroxide; M1–M15: Maize field 1–Maize field 15; P1–P4: Pecan orchard 1–Pecan orchard 4.

Table S9 Catalase activity in H4IIE-*luc* cells after exposure to samples (83 mg/mL) for 24 hours.

Sample name	CAT activity (µM H_2O_2/min/mg protein)	*p*-value	d-value
Control			
BC	42.07 ± 28.75	-	-
Mpumalanga province sampling locations			
M1	41.51 ± 24.78	0.993	0.0
M2	44.96 ± 17.89	0.575	0.1
M3	43.00 ± 22.42	0.923	0.0
M4	39.00 ± 8.33	0.978	0.1
M5	35.60 ± 14.71	0.644	0.2
M6	38.62 ± 14.282	0.942	0.1
M7	43.89 ± 19.35	0.643	0.1
M8	47.33 ± 15.42	0.307	0.2
M9	**73.52 ± 26.74	0.004	**1.1**
M10	45.22 ± 16.79	0.356	0.1
M11	37.05 ± 32.06	0.497	0.2
Vaalharts Valley, Northern Cape province sampling locations			
M12	*64.39 ± 25.98	0.017	**0.8**
M13	53.09 ± 20.18	0.113	0.4
M14	*58.96 ± 16.13	0.017	0.6
M15	61.81 ± 42.78	0.254	0.7
P1	*59.37 ± 21.54	0.030	0.6
P2	49.19 ± 21.22	0.266	0.2
P3	**63.11 ± 18.88	0.005	0.7
P4	39.70 ± 32.98	0.765	0.1

Data is presented as the mean ± standard deviation; asterisks indicate statistically significant differences compared to the blank control (untreated cells) (*$p \leq 0.05$ and **$p \leq 0.01$); values in bold indicate practically significant differences compared to the control (untreated cells) ($d \geq 0.8$); BC: blank control; CAT: catalase; H_2O_2: hydrogen peroxide; M1–M15: Maize field 1–Maize field 15; P1–P4: Pecan orchard 1–Pecan orchard 4.

Table S10 Lipid peroxidation as malondialdehyde (MDA) content (TBARS assay) in HuTu-80 cells after exposure to samples (83 mg/mL) for 24 hours.

Sample name	MDA content (Absorbance)	*p*-value	d-value
Controls			
BC	0.059 ± 0.035	-	-
TMP 1 (240 mM)	**0.310 ± 0.034	0.004	**7.2**
TMP 2 (210 mM)	**0.261 ± 0.016	0.005	**5.8**
TMP 3 (180 mM)	**0.252 ± 0.017	0.006	**5.6**
TMP 4 (150 mM)	**0.193 ± 0.053	0.006	**3.9**
TMP 5 (120 mM)	**0.191 ± 0.027	0.006	**3.8**
TMP 6 (90 mM)	**0.146 ± 0.024	0.006	**2.5**
TMP 7 (60 mM)	**0.130 ± 0.012	0.006	**2.1**
Mpumalanga province sampling locations			
M1	0.052 ± 0.008	0.577	0.2
M2	0.053 ± 0.008	0.889	0.2
M3	0.050 ± 0.006	0.371	0.3
M4	0.052 ± 0.009	0.464	0.2
M5	0.049 ± 0.004	0.269	0.3
M6	0.051 ± 0.004	0.798	0.2
M7	0.054 ± 0.004	0.403	0.1
M8	0.055 ± 0.006	0.500	0.1
M9	0.054 ± 0.005	0.423	0.1
M10	0.050 ± 0.005	0.423	0.3
M11	0.050 ± 0.004	0.577	0.2
Vaalharts Valley, Northern Cape province sampling locations			
M12	0.058 ± 0.010	0.119	0.0
M13	0.051 ± 0.005	0.601	0.2
M14	0.048 ± 0.001	0.101	0.3
M15	0.050 ± 0.002	0.493	0.3
P1	0.063 ± 0.022	0.156	0.1
P2	0.062 ± 0.019	0.178	0.1
P3	0.060 ± 0.012	0.131	0.0
P4	0.073 ± 0.037	0.143	0.4

Data is presented as the mean ± standard deviation; asterisks indicate statistically significant differences compared to the blank control (untreated cells) (*$p \leq 0.05$ and **$p \leq 0.01$); values in bold indicate practically significant differences compared to the control (untreated cells) ($d \geq 0.8$); BC: blank control; M1–M15: Maize field 1–Maize field 15; MDA: malondialdehyde; P1–P4: Pecan orchard 1–Pecan orchard 4: TBARS: thiobarbituric acid reactive substance; TMP: 1,1,3,3-tetramethoxypropane.

Table S11 Lipid peroxidation as malondialdehyde (MDA) content (TBARS assay) in H4IIE-*luc* cells after exposure to samples (83 mg/mL) for 24 hours.

Sample name	MDA content (Absorbance)	*p*-value	d-value
Controls			
BC	0.061 ± 0.029	-	-
TMP 1 (240 mM)	**0.310 ± 0.034	0.004	**8.7**
TMP 2 (210 mM)	**0.261 ± 0.016	0.004	**7.0**
TMP 3 (180 mM)	**0.252 ± 0.017	0.004	**6.7**
TMP 4 (150 mM)	**0.193 ± 0.053	0.005	**4.6**
TMP 5 (120 mM)	**0.191 ± 0.027	0.005	**4.6**
TMP 6 (90 mM)	**0.146 ± 0.024	0.008	**3.0**
TMP 7 (60 mM)	**0.130 ± 0.012	0.010	**2.4**
Mpumalanga province sampling locations			
M1	0.057 ± 0.017	0.907	0.1
M2	0.056 ± 0.014	0.538	0.2
M3	0.059 ± 0.016	0.301	0.1
M4	0.049 ± 0.006	0.340	0.4
M5	0.049 ± 0.005	0.736	0.4
M6	0.047 ± 0.004	0.259	0.5
M7	0.055 ± 0.028	0.133	0.2
M8	0.049 ± 0.005	0.736	0.4
M9	0.068 ± 0.033	0.166	0.2
M10	*0.069 ± 0.026	0.030	0.3
M11	0.054 ± 0.005	0.275	0.3
Vaalharts Valley, Northern Cape province sampling locations			
M12	0.053 ± 0.003	0.270	0.3
M13	0.052 ± 0.004	0.371	0.3
M14	0.054 ± 0.004	0.218	0.2
M15	0.053 ± 0.002	0.260	0.3
P1	0.055 ± 0.012	0.260	0.2
P2	0.057 ± 0.008	0.241	0.1
P3	*0.062 ± 0.008	0.034	0.0
P4	0.058 ± 0.018	0.0163	0.1

Data is presented as the mean ± standard deviation; asterisks indicate statistically significant differences compared to the blank control (untreated cells) (*$p \leq 0.05$ and **$p \leq 0.01$); values in bold indicate practically significant differences compared to the control (untreated cells) ($d \geq 0.8$); BC: blank control; M1–M15: Maize field 1–Maize field 15; MDA: malondialdehyde; P1–P4: Pecan orchard 1–Pecan orchard 4: TBARS: thiobarbituric acid reactive substance; TMP: 1,1,3,3-tetramethoxypropane.

Table S12 Acetylcholinesterase activity in H4IIE-*luc* cells after exposure to samples (83 mg/mL) for 24 hours.

Sample name	AChE activity (x 10^{-4} absorbance/min/mg protein)	*p*-value	d-value
Control			
BC	4.78 ± 7.57	-	-
Mpumalanga province sampling locations			
M1	2.57 ± 0.93	0.896	0.3
M2	2.44 ± 0.54	0.906	0.3
M3	1.61 ± 0.53	0.090	0.4
M4	5.98 ± 7.62	0.281	0.2
M5	1.82 ± 0.99	0.139	0.4
M6	4.41 ± 1.42	0.106	0.0
M7	**7.50 ± 4.45	0.008	0.4
M8	3.92 ± 1.87	0.228	0.1
M9	3.03 ± 2.13	0.896	0.2
M10	**0.97 ± 0.67	0.001	0.5
M11	1.48 ± 0.61	0.059	0.4
Vaalharts Valley, Northern Cape province sampling locations			
M12	**1.13 ± 0.58	0.003	0.5
M13	*1.52 ± 1.02	0.044	0.4
M14	*1.66 ± 1.28	0.047	0.4
M15	2.91 ± 3.41	0.439	0.2
P1	1.66 ± 0.88	0.125	0.4
P2	2.41 ± 1.75	0.484	0.3
P3	5.97 ± 12.00	0.239	0.2
P4	3.40 ± 3.51	0.427	0.2

Data is presented as the mean ± standard deviation; asterisks indicate statistically significant differences compared to the blank control (untreated cells) (*$p \leq 0.05$ and **$p \leq 0.01$); values in bold indicate practically significant differences compared to the control (untreated cells) ($d \geq 0.8$); AChE: acetylcholinesterase; BC: blank control; M1–M15: Maize field 1–Maize field 15; P1–P4: Pecan orchard 1–Pecan orchard 4.

Table S13 Agrochemicals detected in maize field and pecan orchard soil samples following chemical screening (positive ionisation). Arranged according to their abundance.

Nr	Class	Name	Abundance	Score	m/z	RT (min)
Maize 1						
1	Herbicide	Tepraloxydim	266651	65.63	359.1747	5.286
2	Insecticide	Phosphamidon	242862	61.02	317.103	14.879
3	Insecticide	Fenpropathrin	192688	72.34	350.1779	0.938
4	Insecticide	Carbosulfan	150854	54.81	403.1998	5.585
5	Herbicide	Trifluralin	63304	88.68	336.1159	13.030
6	Fungicide	Nuarimol	50156	59.31	332.0942	13.130
7	Insecticide	Malaoxon	50156	72.57	332.0942	13.130
8	Insecticide	Dinocap	30616	83.21	365.1687	0.838
9	Herbicide	Sethoxydim	24852	67.99	345.2221	0.938
10	Herbicide	MCPA-thioethyl	22670	85.52	245.0414	13.979
11	Pesticide	Maleic hydrazide (MH)	17854	58.11	130.0611	0.638
12	Herbicide	Naptalam	16930	52.92	292.1007	15.078
13	Herbicide	Fluazifop-butyl	16006	60.78	406.1211	14.179
14	Insecticide	Mipafox	15475	74.93	205.0862	1.138
15	Fungicide	Pyrazophos	9417	53.49	374.095	14.179
16	Insecticide	Cevadin	9340	82.88	614.3294	5.435
17	Herbicide	Piperophos	9308	54.84	371.1606	5.136
18	Herbicide	Metribuzin	8527	60.20	232.1225	5.435
19	Insecticide	Lethane 384	8306	90.86	221.1325	1.837
20	Insecticide	Jasmolin I	7600	86.79	353.2079	1.837
21	Fungicide	Thiophanate-methyl	7233	79.66	365.0335	13.030
22	Herbicide	Dicryl	6917	53.29	247.039	13.979
23	Insecticide	Tebupirimfos	6361	54.72	319.1255	0.788
24	Pesticide	Diphenylamine	6059	76.47	170.0983	5.236
25	Herbicide	Lambast	5943	67.55	324.1434	14.029
26	Insecticide	Methiocarb	5928	60.94	248.0717	14.879
27	Insecticide	Bromophos-ethyl	5725	57.27	416.8683	0.438
28	Fungicide	Propamocarb	5723	77.54	189.1583	5.286
29	Herbicide	Penoxalin	5574	65.71	304.1263	3.286
30	Insecticide	Bendiocarb	5472	56.59	241.1166	15.929
31	Herbicide	Pretilachlor	5444	65.78	329.1983	0.938
32	Insecticide	Jasmolin I	5300	91.43	353.2081	5.286
33	Insecticide	Phoxim	5295	69.62	299.0593	7.084
34	Insecticide	Jasmolin I	5220	75.13	353.2083	2.387
35	Insecticide	Cartap	5076	64.16	255.0942	15.978
36	Insecticide	Jasmolin I	4980	81.03	353.2078	1.438
37	Insecticide	Thanite	4646	94.07	271.1468	16.128
38	Fungicide	Bupirimate	4336	65.46	317.1641	15.779
39	Insecticide	Fenamiphos	4088	75.13	321.1414	0.888
40	Insecticide	Demeton-S-methylsulfone	3970	59.43	280.0428	0.938
41	Fungicide	Diniconazole	3933	52.80	343.1097	13.879

Nr	Class	Name	Abundance	Score	m/z	RT (min)
42	Herbicide	Fluridone	3716	58.48	352.0891	13.080
43	Insecticide	Jasmolin I	3662	75.86	353.2079	3.186
44	Herbicide	Furilazole	3545	63.63	278.0341	2.087
45	Fungicide	Bupirimate	3539	68.70	317.1622	13.580
46	Insecticide	Dicrotophos Bildrin	3503	66.01	255.1101	15.029
47	Insecticide	Flucythrinate	3276	59.66	469.1946	1.238
48	Insecticide	Isofenphos	3194	70.74	363.1502	0.888
49	Insecticide	Benfuracarb	3142	77.78	433.1746	20.976
50	Herbicide	Imazamox	3129	71.34	323.171	13.530
51	Herbicide	Lenacil	2975	60.95	257.1282	1.937
52	Herbicide	Flurtamone	2960	81.56	334.1039	7.234
53	Herbicide	Cinidon-ethyl	2889	68.07	411.0877	0.488
54	Herbicide	Atrazine-desethyl	2820	51.86	188.0669	1.238
55	Herbicide	Imazapyr	2814	61.69	279.147	13.180
56	Herbicide	Ethofumesate	2790	65.20	304.1216	15.929
57	Insecticide	Fenazaquin	2760	83.59	307.1798	13.030
58	Herbicide	Ethalfluralin	2732	57.40	334.1039	7.234
59	Insecticide	Jasmolin I	2598	79.36	353.2078	1.138
60	Herbicide	Benoxacor	2564	68.62	282.0063	0.638
61	Fungicide	Benomyl	2557	67.41	291.1453	13.180
62	Insecticide	Diazinon (Dimpylate)	2545	59.08	322.1317	0.788
63	Herbicide	Fluometuron	2521	76.54	250.1163	16.078
64	Fungicide	Fenamidone	2436	64.25	334.0995	13.130
65	Insecticide	Dinocap	2389	52.13	365.1675	13.180
66	Pesticide	Ancymidol	2278	84.95	257.1278	1.937
67	Herbicide	Allidochlor	2265	61.00	174.0673	1.837
68	Herbicide	Methoxyphenone	2194	64.05	241.1241	1.837
69	Insecticide	Demeton-S-methylsulfoxide	2168	64.80	269.0046	0.938
70	Herbicide	Imazaquin	2029	62.73	329.1607	15.879
71	Herbicide	Simazine	1917	69.87	219.1123	0.888
72	Herbicide	Buminafos	1847	82.53	370.249	5.635
73	Insecticide	Tebufenozide	1847	82.14	370.249	5.685
74	Insecticide	Formetanate	1829	57.31	244.1055	7.534
75	Fungicide	Triflumizol	1815	68.40	368.0739	5.635
76	Insecticide	Jasmolin I	1813	86.34	353.2076	4.335
77	Fungicide	Dichlobutrazol	1786	54.69	345.1247	14.029
78	Herbicide	Sulfometuron-methyl	1759	58.47	365.0907	13.929
79	Fungicide	Triamiphos	1738	67.88	295.1444	5.735
80	Insecticide	Tebufenozide	1689	50.20	353.2234	13.280
81	Fungicide	Picoxystrobin	1666	58.17	385.1394	6.385
82	Herbicide	Imazethapyr	1656	52.91	307.1776	15.978
83	Fungicide	Isazofos	1549	61.56	331.074	7.134
84	Fungicide	Benomyl	1497	71.95	291.1459	5.685
85	Herbicide	Nitraline	1467	60.08	368.0863	14.829

Nr	Class	Name	Abundance	Score	m/z	RT (min)
86	Fungicide	Prothiocarb	1400	55.95	213.1017	1.188
87	Fungicide	Pencycuron	1364	67.16	351.1229	20.976
88	Insecticide	Ethoprofos	1351	72.86	260.0927	5.535
89	Fungicide	Kresoxim-methyl	1321	55.97	331.1672	5.186
90	Herbicide	Aziprotryne	1320	59.96	226.0875	15.879
91	Fungicide	Hexaconazole	1279	57.93	336.0643	13.130
92	Insecticide	Cyromazine	1261	68.79	189.0866	8.383
93	Herbicide	Picloram	1221	69.65	257.9611	0.488
94	Insecticide	Tebufenozide	1199	56.49	370.2504	9.133
95	Herbicide	Cyprazine	1190	55.38	245.129	2.687
96	Insecticide	Allethrin II	1133	67.88	347.1828	14.629
97	Herbicide	Chlorpropham	1120	56.35	231.0906	15.479
98	Insecticide	Tebufenozide	1107	77.03	370.2496	6.735
99	Fungicide	Fuberidazole	1037	78.43	185.0717	6.735
Maize 2						
1	Fungicide	Flusilazol	376702	72.51	314.0954	3.838
2	Herbicide	Tepraloxydim	199526	66.32	359.1746	5.283
3	Herbicide	Hexazinone	146815	81.24	311.1703	6.945
4	Insecticide	Cevadin	133511	83.33	592.3488	4.333
5	Insecticide	Carbosulfan	111871	57.48	403.2001	5.583
6	Insecticide	Cevadin	101587	59.41	636.3341	3.238
7	Insecticide	Fenpropathrin	96211	70.79	350.1784	0.935
8	Herbicide	Flazasulfuron	83618	79.59	408.0599	15.926
9	Insecticide	Cevadin	61343	59.93	636.3339	3.247
10	Acaricide	Fenpyroximate	58739	55.54	439.2307	1.435
11	Insecticide	Spinosyn A	44448	65.33	749.4892	16.126
12	Insecticide	Pyrethrin I	38978	51.54	351.1906	5.283
13	Herbicide	Medinoterbacetate	30286	80.70	341.0963	0.649
14	Herbicide	Dinoseb-acetate	27288	80.41	341.0963	0.649
15	Herbicide	Sethoxydim	27016	80.87	345.2229	0.935
16	Fungicide	Flusilazol	26777	77.13	314.0945	3.847
17	Herbicide	Diflufenican	26437	82.01	439.0704	0.599
18	Herbicide	Fluazifop-butyl	23402	72.04	406.1214	14.177
19	Herbicide	Trifluralin	18194	81.70	336.1158	13.028
20	Herbicide	Diflufenican	17774	67.34	395.085	5.533
21	Insecticide	Bendiocarb	17228	59.25	241.1166	15.926
22	Insecticide	Terbufos	17066	55.17	306.079	9.830
23	Insecticide	Dinocap	16927	93.97	365.1694	0.835
24	Herbicide	Medinoterbacetate	16062	78.64	341.0962	0.491
25	Insecticide	Isolan	12903	87.03	210.1266	3.097
26	Fungicide	Pyrazophos	12482	78.00	374.095	14.177
27	Insecticide	Demeton-O	11998	87.50	257.0444	3.138
28	Herbicide	Clethodim	9558	53.81	377.1684	1.585
29	Insecticide	Etrimfos	9442	50.67	291.0544	6.995

Nr	Class	Name	Abundance	Score	m/z	RT (min)
30	Herbicide	Diethofencarb	9031	74.38	266.1404	7.286
31	Pesticide	2,4-Dinitrophenol	8182	62.54	183.0036	7.836
32	Herbicide	Pretilachlor	8150	70.36	329.1989	0.935
33	Insecticide	Tebufenozide	7741	78.47	397.2128	7.486
34	Insecticide	Pyrimitate	7674	64.45	328.0828	15.976
35	Fungicide	Furmecyclox	7494	54.30	250.1444	7.036
36	Herbicide	Florasulam	7183	59.78	404.0261	7.186
37	Herbicide	Pyriftalid	7150	53.58	336.1037	10.180
38	Herbicide	Profluralin	7138	77.76	348.1156	13.128
39	Acaricide	Aminocarb	6852	92.68	267.1364	7.944
40	Insecticide	Lethane 384	6383	90.89	221.1319	1.435
41	Herbicide	MCPA-thioethyl	6199	70.81	245.0414	13.977
42	Insecticide	Disulfoton	6076	59.26	273.0226	1.840
43	Insecticide	Pyrimitate	6017	73.10	328.0847	16.476
44	Insecticide	Tebufenozide	5957	82.05	397.2131	7.445
45	Herbicide	Metribuzin	5873	50.05	232.1211	5.433
46	Herbicide	Diethofencarb	5573	58.04	266.1403	7.345
47	Insecticide	Jasmolin I	5446	94.51	353.2079	1.835
48	Insecticide	Fenazaquin	5268	81.26	351.1725	7.345
49	Herbicide	Cyprazine	5267	59.98	286.1082	7.386
50	Insecticide	Cyromazine	5150	65.21	184.1325	0.786
51	Insecticide	Fenazaquin	5147	80.04	365.1868	6.986
52	Fungicide	Thiophanate-methyl	4693	78.00	365.0336	13.028
53	Herbicide	Penoxalin	4420	73.40	304.1272	3.284
54	Insecticide	Bomyl	4347	53.52	327.0505	4.846
55	Pesticide	4,4'-Bipyridyl	4017	82.51	215.0828	3.138
56	Pesticide	2,4-Dinitrophenol	3970	58.05	183.0036	7.045
57	Herbicide	Diethofencarb	3920	59.48	266.1403	4.038
58	Herbicide	Simetryn	3671	60.45	212.0986	6.545
59	Herbicide	Diallate	3640	51.06	287.0747	15.926
60	Insecticide	Jasmolin I	3603	77.75	353.208	2.384
61	Insecticide	Bulan	3549	69.46	346.0381	15.026
62	Acaricide	Aminocarb	3416	93.68	267.136	7.336
63	Herbicide	Flamprop-isopropyl	3406	59.80	364.109	7.032
64	Acaricide	Methiocarb-sulfoxide	3190	59.83	259.1096	0.686
65	Insecticide	Fluenethyl	3190	54.84	259.1096	0.686
66	Herbicide	Cyprazine	3149	57.94	286.1082	7.345
67	Herbicide	Cyanazine	3133	54.51	258.1228	16.076
68	Fungicide	2,3-Dichlorophenol	3090	54.62	206.9632	8.135
69	Pesticide	Maleic hydrazide (MH)	2976	75.17	130.0611	0.686
70	Fungicide	Bupirimate	2934	73.14	317.1636	15.776
71	Acaricide	Aminocarb	2897	76.12	267.1363	7.045
72	Insecticide	Jasmolin I	2790	73.31	353.2068	5.283
73	Insecticide	Jasmolin I	2788	88.87	353.2084	3.184

Nr	Class	Name	Abundance	Score	m/z	RT (min)
74	Herbicide	Pebulate	2715	83.34	226.1232	0.636
75	pesticide	4,4'-Bipyridyl	2675	75.42	215.0811	3.097
76	Herbicide	Desmedipham	2668	71.33	359.1244	7.095
77	Fungicide	Picoxystrobin	2664	75.45	385.1392	13.078
78	Herbicide	Fluroxypyr-meptyl	2471	52.04	367.0999	0.536
79	Herbicide	Dinitramine	2445	54.85	340.1214	15.876
80	Insecticide	MNFA	2355	77.88	235.1238	13.128
81	Insecticide	Carbaryl	2260	52.29	219.1147	0.686
82	Insecticide	Jasmolin I	2247	76.61	353.2076	1.435
83	Acaricide	Aminocarb	2147	61.83	267.137	4.388
84	Fungicide	Quassin	2077	63.17	389.198	6.836
85	Insecticide	Thiofanox	2068	55.10	219.115	0.885
86	Insecticide	Fonofos	2030	65.43	247.039	13.977
87	Fungicide	2,3-Dichlorophenol	2010	50.71	206.9636	5.388
88	Herbicide	Chlorbromuron	1964	61.38	294.9652	2.934
89	Herbicide	Imazamox	1950	70.15	323.1725	13.427
90	Insecticide	Mecarbam	1936	65.31	330.0615	15.076
91	Insecticide	Flucythrinate	1903	66.52	469.1937	1.285
92	Fungicide	Benomyl	1884	81.45	349.1524	6.936
93	Insecticide	Allethrin II	1834	69.71	391.1736	6.945
94	pesticide	4,4'-Bipyridyl	1822	60.34	157.0775	0.835
95	Herbicide	Imazapyr	1794	58.10	279.1479	13.827
96	Herbicide	Dinoseb-acetate	1773	67.84	341.0988	7.886
97	Insecticide	Deguelin	1769	72.54	412.1773	1.135
98	Insecticide	Nitenpyram	1721	52.22	271.0929	15.926
99	Insecticide	Diflubenzuron	1718	50.66	328.0658	15.076
100	Herbicide	Endothal	1690	84.81	209.0427	2.184
101	Insecticide	Diazinon (Dimpylate)	1681	54.02	322.136	0.786
102	Herbicide	Glyphosate	1669	64.58	228.0296	3.088
103	Fungicide	2,3-Dichlorophenol	1662	55.29	206.9633	3.488
104	Insecticide	Triflumuron	1656	51.80	357.0282	7.186
105	Plant growth regulator	Trinexapac-ethyl	1647	56.67	251.0949	3.597
106	Herbicide	Naptalam	1638	66.26	309.1245	13.078
107	Herbicide	Dicryl	1624	56.12	247.039	13.977
108	Insecticide	Fenazaquin	1580	58.12	307.1794	13.777
109	Herbicide	Bentazone	1575	58.32	239.0493	7.336
110	Herbicide	Atrazine-desethyl-desisopropyl	1552	59.52	204.0294	3.247
111	Herbicide	Piperophos	1543	70.03	371.1574	13.427
112	Insecticide	Dinocap	1539	66.03	423.1776	6.995
113	Fungicide	2-Phenylphenol	1536	71.17	169.0647	0.890
114	Herbicide	Difenoxuron	1533	55.41	285.121	3.397
115	Fungicide	Tricyclazole	1531	63.94	190.0437	9.280
116	Herbicide	Isocarbamide	1517	74.60	186.122	2.135
117	Herbicide	Aziprotryne	1506	73.56	226.0862	16.026

Nr	Class	Name	Abundance	Score	m/z	RT (min)
118	Herbicide	Fluometuron	1487	66.72	231.0745	3.147
119	Herbicide	Cyanazine	1454	55.39	239.0804	3.147
120	Insecticide	Quintiofos	1449	51.32	330.0738	14.277
121	Herbicide	Ethiolate	1447	70.95	220.1014	7.086
122	Pesticide	Diphenylamine	1444	68.49	170.0975	1.585
123	Fungicide	Bitertanol	1428	65.02	338.1855	16.026
124	Pesticide	Aldicarb-sulfone (Aldoxycarb)	1426	64.38	245.057	0.985
125	Insecticide	Diafenthiuron	1421	65.64	383.2156	7.186
126	Herbicide	Aziprotryne	1409	60.00	270.0775	5.346
127	Herbicide	Daminozide	1347	77.69	183.0731	2.334
128	Herbicide	Isoxaben	1307	52.70	391.1868	6.836
129	Herbicide	Cyanazine	1297	62.74	239.0826	3.088
130	Herbicide	Picloram	1292	68.46	238.9176	7.786
131	Herbicide	Chloral methyl hemiacetal	1291	66.51	238.9465	6.895
132	Fungicide	Cymoxanil (Curzate)	1259	78.58	257.0899	3.138
133	Fungicide	Prothiocarb	1235	69.21	213.1015	7.981
134	Herbicide	Methoxyphenone	1232	78.57	241.1238	1.835
135	Insecticide	Cartap	1204	56.27	255.093	15.576
136	Herbicide	Profoxydim	1199	50.42	483.2061	10.180
137	Herbicide	EPTC	1198	53.59	234.1149	7.186
138	Fungicide	Myclobutanil	1196	50.23	287.1079	7.395
139	Insecticide	Jasmolin I	1174	91.92	353.2087	4.333
140	Herbicide	Fluchloralin	1163	54.83	356.0596	5.533
141	Fungicide	Methfuroxam	1158	66.07	252.0992	8.031
142	Fungicide	Hexaconazole	1154	57.35	331.1095	16.026
143	Herbicide	Oxadiargyl	1154	51.93	385.0371	6.445
144	Plant growth regulator	Dikegulac	1140	82.76	333.1212	8.044
145	Fungicide	Iprobenfos	1137	63.14	347.1106	3.088
146	Insecticide	Tinox	1129	64.27	275.0181	6.845
147	Insecticide	Demeton-O-methyl	1129	64.27	275.0181	6.845
148	Fungicide	Triamiphos	1123	79.99	293.1293	6.836
149	Insecticide	Benzoximate	1121	65.80	386.0753	5.533
150	Insecticide	Isolan	1117	68.02	212.1375	5.183
151	Herbicide	Erbon	1105	56.04	424.9089	7.145
152	Insecticide	Tetrachlorvinphos	1105	52.92	424.9089	7.145
153	Herbicide	Deiquat	1092	76.08	183.0936	3.497
154	Herbicide	Atrazine-desethyl-desisopropyl	1091	51.49	204.0307	3.288
155	Fungicide	Binapacryl	1088	65.68	345.1046	5.883
156	Herbicide	Morfamquate	1072	61.76	513.2673	6.986
157	Insecticide	Lethane 60	1038	82.82	308.1649	0.835
158	Fungicide	Pyraclostrobin	1036	51.45	405.1331	15.876
159	Insecticide	Dimetilan	1034	80.50	285.1204	3.438
160	Fungicide	Bupirimate	1030	55.82	361.1539	3.647

Nr	Class	Name	Abundance	Score	m/z	RT (min)
161	Herbicide	Metribuzin	1023	71.94	273.1027	4.796
162	Insecticide	Tebufenozide	1021	50.83	375.2012	13.227
163	Insecticide	Demeton-S-methylsulfone	1018	67.57	280.0428	0.935
164	Fungicide	Propamocarb	1017	51.08	189.1583	15.976
165	Herbicide	Deiquat	1007	82.38	183.0936	3.238
166	Insecticide	Etrimfos	1000	58.17	291.0568	6.836
Maize 3						
1	Herbicide	Tepraloxydim	202680	61.99	359.1751	5.284
2	Insecticide	Fenpropathrin	110102	72.09	350.1782	0.936
3	Acaricide	Fenpyroximate	50997	63.52	439.2312	1.436
4	Insecticide	Bendiocarb	39903	63.95	241.1167	15.927
5	Insecticide	Pyrethrin I	38999	54.28	351.1914	5.284
6	Insecticide	Fenamiphos	25286	54.69	321.1402	0.836
7	Insecticide	Dinocap	20737	88.82	365.1688	0.836
8	Insecticide	Quintiofos	17419	69.79	330.0725	14.228
9	Herbicide	Clethodim	12685	53.53	377.1686	1.586
10	Insecticide	Thanite	11652	77.79	271.1476	16.127
11	Insecticide	Spinosyn A	9667	51.42	749.4882	16.127
12	Insecticide	Lethane 384	9659	94.73	221.1328	5.584
13	Herbicide	Flazasulfuron	8545	73.63	408.0602	15.927
14	Herbicide	Pretilachlor	7287	70.35	329.1987	0.936
15	Insecticide	Mipafox	6149	84.78	205.086	1.436
16	Insecticide	Isofenphos	5192	61.66	363.1526	0.936
17	Herbicide	Cinidon-ethyl	4949	64.67	411.089	0.487
18	Insecticide	Pymetrozine	4329	72.19	218.1025	0.637
19	Insecticide	Jasmolin I	4329	70.86	353.2091	2.385
20	Insecticide	Jasmolin I	4262	76.50	353.2088	1.836
21	Herbicide	Buturon	3867	55.47	254.1059	15.027
22	Acaricide	Methiocarb-sulfoxide	3774	61.88	259.1089	0.687
23	Herbicide	Penoxalin	3568	60.38	304.1269	3.185
24	Herbicide	Metribuzin	3499	90.31	232.1236	5.434
25	Fungicide	Isazofos	3423	64.42	331.0764	14.228
26	Fungicide	Furmecyclox	3400	51.73	252.1609	14.827
27	Insecticide	Jasmolin I	2906	96.68	353.2086	5.434
28	Insecticide	Deguelin	2792	87.83	412.1777	1.086
29	Fungicide	Bupirimate	2524	65.37	317.1641	15.777
30	Fungicide	Bupirimate	2492	68.68	317.164	13.578
31	Insecticide	Barthrin	2455	64.48	337.1219	0.736
32	Insecticide	Jasmolin I	2435	83.19	353.2087	3.185
33	Insecticide	Flucythrinate	2406	75.13	469.1946	1.236
34	Insecticide	MNFA	2344	84.07	235.1246	13.128
35	Fungicide	N,N-Dimethyl-N'-p-tolylsulfonyldiamide (DMST)	2333	57.67	215.0829	0.637
36	Herbicide	Naptalam	2277	67.21	309.1242	13.078

Nr	Class	Name	Abundance	Score	m/z	RT (min)
37	Insecticide	Bromophos-ethyl	2218	57.00	416.8668	0.487
38	Insecticide	Prothoate	2018	55.19	303.0975	0.986
39	Insecticide	Cyromazine	1999	60.37	167.1017	15.877
40	Insecticide	Cevadin	1820	89.94	609.3758	5.284
41	Insecticide	Jasmolin I	1806	78.61	353.2073	1.436
42	Insecticide	Jasmolin I	1769	66.81	353.2087	1.136
43	Insecticide	Fenazaquin	1728	85.37	307.1801	13.028
44	Herbicide	Imazamox	1712	78.99	323.1719	13.378
45	Herbicide	Piperophos	1635	80.91	371.1597	15.777
46	Herbicide	Methoxyphenone	1614	56.82	263.1027	5.284
47	Fungicide	Triamiphos	1476	77.59	295.1423	13.128
48	Insecticide	Jasmolin I	1421	62.00	353.2083	4.284
49	Insecticide	Pyrimitate	1345	52.30	323.1286	0.786
50	Herbicide	Profluralin	1342	78.36	365.1421	1.386
51	Herbicide	Ioxynil	1286	55.04	388.8648	0.437
52	Plant growth regulator	Azoluron	1270	73.19	253.1054	13.328
53	Herbicide	Trifluralin	1202	50.72	336.1181	13.028
54	Insecticide	Crufomat	1162	53.14	309.1174	15.027
55	Herbicide	Lenacil	1139	57.24	257.1282	1.936
56	Insecticide	Diazinon (Dimpylate)	1123	71.85	305.1105	0.786
57	Pesticide	Pyridaben	1103	67.04	365.1413	13.428
58	Herbicide	Diethofencarb	1089	64.60	285.1828	15.827
59	Insecticide	Phosphamidon	1056	55.07	317.1039	14.877
Maize 4						
1	Herbicide	Tepraloxydim	154502	63.66	359.1749	5.283
2	Herbicide	Buturon	135636	57.46	254.1062	15.027
3	Herbicide	Flazasulfuron	123817	77.75	408.0597	15.927
4	Herbicide	Chloroxuron	99213	52.72	308.1134	15.027
5	Insecticide	Carbosulfan	89982	51.66	403.2008	5.583
6	Insecticide	Fenpropathrin	74546	68.56	350.1784	0.936
7	Insecticide	Quintiofos	48279	69.64	330.0727	14.227
8	Acaricide	Fenpyroximate	46274	61.54	439.2313	1.435
9	Insecticide	Pyrethrin I	38602	55.33	351.1911	5.283
10	Insecticide	Pirimiphos-ethyl	30518	82.12	334.1367	8.081
11	Fungicide	Triflumizol	24224	67.25	368.0756	5.633
12	Insecticide	Dicrotophos Bildrin	18042	94.44	255.1093	15.027
13	Herbicide	Flurtamone	17458	72.94	351.1346	13.028
14	Herbicide	Sethoxydim	10740	75.60	345.2234	0.936
15	Fungicide	Isazofos	8530	65.56	331.076	14.227
16	Insecticide	Mipafox	7677	78.68	205.086	1.136
17	Herbicide	EPTC	6872	73.81	207.1517	8.281
18	Herbicide	Isoxaflutole	6679	55.69	360.0541	15.027
19	Insecticide	Lethane 384	6258	94.71	221.1324	1.435
20	Insecticide	Bendiocarb	6075	55.68	241.1167	15.927

Nr	Class	Name	Abundance	Score	m/z	RT (min)
21	Insecticide	Cevadin	5884	92.36	614.3304	5.433
22	Insecticide	Thiometon	4897	74.27	264.0323	0.786
23	Herbicide	Methoprotryne	4521	51.54	272.155	13.728
24	Herbicide	Metribuzin	4148	56.39	232.123	5.433
25	Herbicide	Penoxalin	3870	78.01	304.1269	3.134
26	Herbicide	Dinoseb-acetate	3850	72.16	283.0911	13.878
27	Herbicide	Trifloxysulfuron	3845	88.41	438.07	15.877
28	Fungicide	Cyazofamid	3206	55.16	342.0799	15.027
29	Insecticide	Jasmolin I	3204	92.84	353.2082	1.785
30	Herbicide	Pretilachlor	3000	70.79	329.1987	0.936
31	Herbicide	Piperophos	2956	78.92	371.1613	5.134
32	Herbicide	Fluazifop-butyl	2896	65.64	406.1209	14.177
33	Insecticide	Cyromazine	2833	65.17	184.1328	0.836
34	Fungicide	Pyrazophos	2669	62.99	374.0956	14.177
35	Fungicide	Propiconazole	2640	65.60	342.0798	15.027
36	Insecticide	Jasmolin I	2621	75.82	353.2081	2.385
37	Insecticide	MNFA	2480	77.17	235.1247	13.178
38	Insecticide	Jasmolin I	2475	88.81	353.2067	5.433
39	Insecticide	Tebufenozide	2378	60.33	375.2026	13.228
40	Insecticide	Jasmolin I	2316	69.00	353.2082	1.136
41	Herbicide	Imazamox	2298	76.38	323.1727	13.678
42	Insecticide	Tebufenozide	2225	59.39	375.2012	15.877
43	Fungicide	Triamiphos	2160	91.46	295.144	5.533
44	Fungicide	Bupirimate	2118	62.52	317.1637	15.527
45	Insecticide	Isofenphos	2059	50.69	368.1069	13.028
46	Herbicide	Cloquintocet-mexyl	2056	51.62	336.1372	8.081
47	Fungicide	Bupirimate	2043	64.28	317.164	13.928
48	Herbicide	Dinitramine	1973	91.24	323.0965	9.380
49	Fungicide	Propamocarb	1948	83.48	189.158	5.233
50	Insecticide	Jasmolin I	1946	57.35	353.209	13.378
51	Insecticide	Thanite	1912	58.25	271.1495	16.127
52	Insecticide	Deguelin	1905	86.90	412.1762	1.136
53	Herbicide	Lenacil	1893	67.84	235.1457	11.130
54	Herbicide	Orbencarb	1886	59.20	258.0693	5.933
55	Fungicide	Fenamidone	1826	80.16	334.1009	7.282
56	Herbicide	Desmedipham	1818	55.42	323.0966	9.380
57	Insecticide	Jasmolin I	1803	83.72	353.2078	1.435
58	Pesticide	Maleic hydrazide (MH)	1771	57.14	130.0612	0.686
59	Insecticide	Sulfirame	1762	61.32	282.1144	15.927
60	insecticide	Methoxyfenozide	1759	54.53	391.194	13.528
61	Insecticide	Fenazaquin	1697	90.10	307.1806	9.880
62	Herbicide	Difenzoquate	1611	70.02	267.1718	11.180
63	Insecticide	Carbaryl	1600	56.37	219.1124	1.336
64	Herbicide	Clethodim	1557	59.59	377.169	1.735

Nr	Class	Name	Abundance	Score	m/z	RT (min)
65	Fungicide	Picoxystrobin	1544	60.09	368.1069	13.028
66	Insecticide	Carbaryl	1507	70.45	219.1146	2.335
67	Herbicide	Benoxacor	1441	57.39	282.0067	0.586
68	Insecticide	Coumaphos	1431	69.88	380.0485	13.728
69	Herbicide	Karbutilate	1347	54.85	297.1941	13.128
70	Herbicide	Cinidon-ethyl	1346	64.26	411.0888	0.586
71	Plant growth regulator	Azoluron	1314	56.09	253.1049	13.328
72	Acaricide	Aminocarb	1265	62.61	231.113	0.886
73	Insecticide	Jasmolin I	1252	80.77	353.2073	3.184
74	Herbicide	Metobromuron	1206	51.97	276.0331	0.936
75	Herbicide	Daminozide	1203	76.62	183.0735	1.785
76	Plant growth regulator	Dikegulac	1183	65.25	275.1141	7.282
77	Herbicide	Picloram	1175	50.21	257.9624	0.586
78	Insecticide	Phenthoate	1170	56.14	338.0657	9.380
79	Pesticide	Aldicarb-sulfone (Aldoxycarb)	1168	71.83	245.0578	0.936
80	Herbicide	Chlorbromuron	1157	50.83	294.965	2.984
81	Insecticide	Demeton-S-methylsulfone	1157	59.02	280.0448	0.936
82	Insecticide	Hydramethylnon	1122	51.97	517.1877	1.435
83	Insecticide	Bomyl	1120	66.21	305.037	9.980
84	Insecticide	Fenamiphos	1094	62.65	304.1142	16.027
85	Pesticide	Ancymidol	1082	79.13	257.1286	1.935
86	Herbicide	Ethalfluralin	1079	80.82	334.102	7.282
87	Insecticide	Etrimfos	1064	60.25	293.0704	13.778
88	Herbicide	Prodiamine	1045	72.30	351.1261	11.180
89	Fungicide	Dimoxystrobin	1023	58.08	327.17	13.128
90	Herbicide	Methoxyphenone	1016	88.73	241.1224	1.785
91	Herbicide	Dicryl	1012	58.99	247.0403	13.928
92	Pesticide	Thiofanox-sulphoxide	1002	50.35	257.0926	3.834
Maize 5						
1	Herbicide	Tepraloxydim	157025	61.82	359.1751	5.245
2	Herbicide	Trifluralin	92222	83.36	336.1153	13.040
3	Herbicide	Flazasulfuron	81104	77.97	408.0598	15.938
4	Herbicide	Sethoxydim	80863	63.83	350.1783	0.898
5	Herbicide	Neburon	41531	55.24	292.1001	15.038
6	Insecticide	Pyrethrin I	38611	50.65	351.1912	5.245
7	Insecticide	Dinocap	17969	76.28	365.1688	0.848
8	Insecticide	Barthrin	16942	52.81	337.1194	13.040
9	Insecticide	Terbufos	11583	62.97	306.0789	9.792
10	Herbicide	Fluridone	10429	64.29	352.0889	13.040
11	Insecticide	Bendiocarb	8702	62.30	241.1164	15.938
12	Insecticide	Thanite	6350	66.13	271.1471	16.138
13	Insecticide	Cevadin	6202	85.48	614.3305	5.245
14	Insecticide	Triazophos	5997	51.67	331.1005	15.938
15	Insecticide	Lethane 384	4705	91.72	221.1326	1.447

Nr	Class	Name	Abundance	Score	m/z	RT (min)
16	Herbicide	Piperophos	4444	73.06	371.1612	5.146
17	Insecticide	Jasmolin I	4365	90.64	353.2085	1.797
18	Herbicide	MCPA-thioethyl	4065	81.22	245.0414	13.989
19	Herbicide	Daminozide	4007	84.26	183.0755	1.098
20	Fungicide	Pyrazophos	3857	72.02	391.1191	15.938
21	Insecticide	Jasmolin I	3398	96.94	353.2081	2.347
22	Insecticide	Mipafox	3134	78.07	205.0862	1.397
23	Herbicide	Metribuzin	3113	73.97	232.1238	5.595
24	Herbicide	Orbencarb	2771	55.59	258.0694	5.945
25	Insecticide	Isofenphos	2753	63.06	363.1524	0.898
26	Insecticide	Diafenthiuron	2748	78.52	385.229	5.595
27	Herbicide	Penoxalin	2742	73.03	304.1271	3.146
28	Insecticide	Phosphamidon	2732	58.45	317.1028	14.839
29	Fungicide	Bupirimate	2635	81.72	317.1628	13.589
30	herbicide	Cinidon-ethyl	2610	65.25	411.0881	0.498
31	Insecticide	Jasmolin I	2326	67.13	353.2071	1.447
32	Fungicide	Bupirimate	2267	85.14	317.164	15.788
33	Insecticide	Deguelin	2253	64.54	412.1762	1.098
34	Herbicide	Difenoxuron	2187	62.84	309.1234	13.090
35	Herbicide	Chlorpropham	2156	55.11	231.0908	15.938
36	Insecticide	Jasmolin I	2112	94.16	353.2081	5.245
37	Herbicide	Imazethapyr	2050	58.36	307.1781	13.290
38	Insecticide	MNFA	2010	81.86	235.1246	13.140
39	Acaricide	Methiocarb-sulfoxide	1935	58.51	259.1086	0.698
40	Insecticide	Monocrotophos	1933	50.81	241.0924	5.745
41	Insecticide	Phoxim	1923	77.63	299.0605	7.044
42	Herbicide	Imazamox	1893	74.55	323.1724	13.440
43	Insecticide	Fenazaquin	1892	65.58	307.1781	13.290
44	Fungicide	Mepanipyrim	1773	53.31	246.1016	15.888
45	Herbicide	Lambast	1726	50.12	324.1431	14.039
46	Herbicide	Naptalam	1587	80.42	309.1234	13.090
47	Insecticide	Flucythrinate	1546	61.44	469.1946	1.298
48	Herbicide	Dicryl	1522	57.66	247.0401	13.989
49	Fungicide	Methfuroxam	1490	54.94	252.0994	8.043
50	Insecticide	Jasmolin I	1435	80.39	353.2081	3.146
51	Herbicide	Diethofencarb	1422	59.54	285.1792	15.838
52	Herbicide	Trifloxysulfuron	1389	59.72	438.0704	15.888
53	Fungicide	Propamocarb	1382	69.96	189.158	15.938
54	Insecticide	Cyromazine	1355	85.91	167.1038	15.888
55	Insecticide	Dicyclanil	1283	68.46	213.0841	13.040
56	Insecticide	Dicyclanil	1279	60.08	213.0869	13.290
57	Herbicide	Imazaquin	1273	69.49	329.158	16.088
58	Fungicide	Diniconazole	1228	66.94	343.1097	13.889
59	Plant growth regulator	Azoluron	1204	73.33	253.1039	13.290

Nr	Class	Name	Abundance	Score	m/z	RT (min)
60	Fungicide	Rubijervine	1178	67.55	431.3641	16.288
61	Herbicide	Dimefuron	1176	61.11	339.1218	13.090
62	Insecticide	Jasmolin I	1127	74.14	353.2074	1.148
63	Insecticide	Prothoate	1114	51.24	303.0988	0.998
64	Insecticide	Thiofanox	1113	51.18	219.1166	0.898
65	Herbicide	Pretilachlor	1093	73.40	329.1986	6.395
66	Herbicide	Flamprop-isopropyl	1073	54.11	364.1108	6.994
67	Fungicide	Prothiocarb	1045	59.58	213.1013	8.043
Maize 6						
1	Herbicide	Flazasulfuron	304078	69.74	408.0609	15.936
2	Herbicide	Buturon	146440	56.21	254.1063	15.036
3	Herbicide	Chloroxuron	126151	53.82	308.1135	15.036
4	herbicide	Tepraloxydim	110100	61.61	359.1752	5.244
5	Insecticide	Fenpropathrin	41731	74.44	350.1779	0.946
6	Fungicide	Triflumizol	34163	70.20	368.0751	5.643
7	Insecticide	Pyrethrin I	30140	56.27	351.1917	5.244
8	Acaricide	Fenpyroximate	25818	62.62	439.2311	1.445
9	Insecticide	Dicrotophos Bildrin	23837	92.80	255.1095	15.036
10	Herbicide	Fluazifop-butyl	18679	65.30	406.1208	14.187
11	Insecticide	Quintiofos	17865	68.89	330.0727	14.237
12	Fungicide	Pyrazophos	10509	82.18	374.0952	14.187
13	Herbicide	Isoxaflutole	10152	61.72	360.0536	15.036
14	Insecticide	Pirimiphos-ethyl	8805	89.12	334.1366	8.091
15	Herbicide	Flurtamone	6725	50.18	351.1354	13.038
16	Herbicide	Trifluralin	6519	83.82	336.116	13.038
17	Herbicide	MCPA-thioethyl	6049	70.76	245.0415	13.987
18	Fungicide	Methfuroxam	6018	67.88	252.0988	15.886
19	Insecticide	Lethane 384	5912	76.95	221.133	5.244
20	Insecticide	Dinocap	4976	71.80	365.1687	0.846
21	Insecticide	Bendiocarb	4902	70.36	241.117	15.936
22	Herbicide	Sethoxydim	4835	79.55	345.2228	0.946
23	Fungicide	Furmecyclox	4423	57.20	252.1602	14.887
24	Herbicide	Piperophos	4420	75.66	371.1613	5.093
25	Fungicide	Propiconazole	4093	55.99	342.0798	15.036
26	Fungicide	Cyazofamid	4093	53.25	342.0798	15.036
27	Herbicide	Penoxalin	4012	62.90	304.1269	3.194
28	Insecticide	Cevadin	3296	86.81	614.3304	5.244
29	Herbicide	Methoprotryne	3295	54.88	272.1553	13.737
30	Fungicide	Bupirimate	2937	70.96	317.1655	15.787
31	Herbicide	Clethodim	2752	61.73	377.1687	1.595
32	Fungicide	Isazofos	2647	63.88	331.0764	14.237
33	Fungicide	Rubijervine	2384	85.70	414.3381	15.437
34	Herbicide	Atraton	2282	60.92	229.1755	16.086
35	Fungicide	Propamocarb	2210	54.76	189.1589	5.244

Nr	Class	Name	Abundance	Score	m/z	RT (min)
36	Herbicide	Mefenpyr-diethyl	2161	57.23	390.0991	12.588
37	Herbicide	Imazamox	2123	57.26	323.1733	13.438
38	Fungicide	Bupirimate	2097	69.09	317.1642	13.588
39	Pesticide	Maleic hydrazide (MH)	1907	69.01	130.0611	0.696
40	Insecticide	Thanite	1839	62.89	271.1478	16.136
41	Insecticide	MNFA	1812	66.70	235.1241	13.937
42	Herbicide	Dinitramine	1760	80.47	323.0956	9.340
43	Insecticide	Jasmolin I	1740	92.39	353.2077	1.845
44	Herbicide	Linuron	1739	62.41	266.0473	1.945
45	Herbicide	Tralkoxydime	1673	55.51	352.1903	5.244
46	Herbicide	Lenacil	1652	58.49	235.1428	10.990
47	Insecticide	Deguelin	1648	57.58	412.176	1.146
48	Insecticide	Isofenphos	1639	65.79	363.1518	0.946
49	Herbicide	Metribuzin	1619	69.68	232.1241	5.393
50	Herbicide	Picolinafen	1601	85.81	377.0908	8.841
51	Insecticide	Jasmolin I	1571	70.10	353.2064	5.244
52	Insecticide	Fenazaquin	1563	80.92	307.1794	13.338
53	Herbicide	Lambast	1484	55.77	324.1434	13.987
54	Herbicide	Imazapyr	1436	52.06	279.1488	13.638
55	Herbicide	Fenoxaprop-ethyl	1405	61.63	384.0567	15.036
56	Herbicide	Dicryl	1364	68.24	247.0395	13.987
57	Herbicide	Difenzoquate	1339	83.84	267.173	11.140
58	Herbicide	Prodiamine	1299	76.56	351.1271	10.190
59	Herbicide	Ethalfluralin	1299	76.56	351.1271	10.240
60	Insecticide	Jasmolin I	1296	73.54	353.2083	2.345
61	Fungicide	Picoxystrobin	1272	63.62	385.1395	5.943
62	Herbicide	Pebulate	1271	65.29	226.1254	0.596
63	Herbicide	Orbencarb	1240	51.39	258.0699	5.893
64	Fungicide	Benomyl	1198	51.17	291.1476	13.588
65	Herbicide	Flufenacet	1193	54.14	386.0554	15.036
66	Herbicide	Pretilachlor	1161	58.57	329.1989	0.946
67	Insecticide	Tebufenozide	1156	61.08	375.2008	13.088
68	Herbicide	Mefenacet	1121	61.28	321.0661	9.940
69	Herbicide	Cyprazine	1120	58.66	245.1287	1.645
70	Insecticide	Flucythrinate	1093	68.32	469.1929	1.246
71	Insecticide	Fonofos	1093	87.51	247.0392	13.987
72	Fungicide	Ditalimfos (Plondrel)	1090	70.95	300.0432	7.542
73	Insecticide	Parathion-methyl	1061	56.88	281.0366	14.137
74	Fungicide	Binapacryl	1042	61.93	323.1222	0.796
Maize 7						
1	Herbicide	Buturon	294174	55.92	254.1064	15.024
2	Herbicide	Chloroxuron	166159	52.27	308.1134	15.024
3	herbicide	Tepraloxydim	133966	62.62	359.175	5.281
4	Insecticide	Fenpropathrin	62583	67.50	350.1785	0.933

Nr	Class	Name	Abundance	Score	m/z	RT (min)
5	Insecticide	Dicrotophos Bildrin	40379	89.65	255.1092	15.024
6	Acaricide	Fenpyroximate	37821	62.75	439.2315	1.433
7	Fungicide	Triflumizol	29135	68.56	368.0752	5.631
8	Insecticide	Pyrethrin I	26353	53.14	351.1912	5.281
9	Insecticide	Pirimiphos-ethyl	19911	89.44	334.1364	8.129
10	Herbicide	Isoxaflutole	18204	63.57	360.0535	15.024
11	Herbicide	MCPA-thioethyl	11895	85.59	245.0415	13.975
12	Insecticide	Spinosyn A	10164	52.61	749.4885	16.124
13	Insecticide	Dinocap	9716	73.12	365.1692	0.833
14	Herbicide	Flurtamone	8385	61.37	351.1345	13.025
15	Herbicide	Sethoxydim	8097	76.14	345.2232	0.933
16	Fungicide	Propiconazole	7730	54.92	342.0799	15.024
17	Fungicide	Cyazofamid	7730	54.73	342.0799	15.024
18	Herbicide	Methoprotryne	6682	56.12	272.155	13.725
19	Insecticide	Thanite	5640	63.08	271.1474	16.124
20	Herbicide	Piperophos	4983	69.25	371.1615	5.080
21	Insecticide	Mipafox	4832	79.54	205.0862	1.133
22	Fungicide	Myclobutanil	4567	50.48	289.1186	0.783
23	Fungicide	Triamiphos	4361	53.76	317.1281	13.825
24	Herbicide	Penoxalin	4306	81.31	304.1272	3.182
25	Insecticide	Fonofos	4134	54.42	247.0385	13.975
26	Herbicide	Clethodim	3559	68.87	377.1694	1.583
27	Herbicide	Dicryl	3550	61.42	247.0389	13.975
28	Fungicide	Pyrazophos	3527	64.89	391.1216	15.924
29	Insecticide	Diafenthiuron	3408	61.64	385.2296	5.581
30	Herbicide	Dinitramine	3140	82.75	323.0956	9.378
31	Insecticide	Isofenphos	2767	60.14	363.1526	0.933
32	Insecticide	Jasmolin I	2718	75.91	353.209	2.332
33	Herbicide	Cinidon-ethyl	2703	57.38	411.0886	0.484
34	Insecticide	Jasmolin I	2662	90.15	353.2087	1.833
35	Herbicide	Fenoxaprop-ethyl	2647	62.42	384.0575	15.024
36	Fungicide	Triadimenol	2527	52.63	296.1189	15.024
37	Herbicide	Metribuzin	2492	74.17	232.1234	5.431
38	Fungicide	Bupirimate	2422	55.56	317.1645	15.774
39	Insecticide	Phenthoate	2354	73.83	338.0662	9.328
40	Herbicide	Pretilachlor	2303	59.44	329.1989	0.933
41	Herbicide	Methabenzthiazuron	2263	54.42	244.0499	13.575
42	Fungicide	Fenamidone	2103	76.47	334.1001	7.879
43	Herbicide	Medinoterbacetate	2066	64.28	319.0895	13.825
44	Insecticide	Parathion-methyl	2037	57.11	281.0373	14.175
45	Herbicide	Trifluralin	2016	75.82	336.1149	13.025
46	Herbicide	Profluralin	2014	76.71	365.1403	2.382
47	Herbicide	Benoxacor	1947	51.60	282.0066	0.534
48	Herbicide	Diallate	1929	50.79	287.0762	15.974

Nr	Class	Name	Abundance	Score	m/z	RT (min)
49	Herbicide	Imazamox	1924	79.14	323.1729	13.475
50	Insecticide	Lethane 384	1923	92.96	221.1324	4.231
51	Pesticide	Ancymidol	1854	76.15	257.1292	1.933
52	Insecticide	Cartap	1845	61.32	255.0937	15.974
53	Insecticide	Jasmolin I	1811	75.36	353.2079	5.431
54	Herbicide	Flazasulfuron	1728	61.07	408.0603	15.924
55	Insecticide	MNFA	1717	85.57	235.1247	13.875
56	Insecticide	Jasmolin I	1658	54.82	353.209	1.433
57	Herbicide	Ethalfluralin	1539	76.52	334.1001	7.879
58	Insecticide	Cevadin	1411	87.88	609.3751	5.431
59	Herbicide	Linuron	1386	63.20	266.0472	1.933
60	Fungicide	Flutriafol	1377	76.21	319.1376	12.976
61	Herbicide	Mefenacet	1374	75.92	321.0668	9.877
62	Insecticide	Jasmolin I	1239	66.85	353.2083	3.182
63	Herbicide	Picolinafen	1216	61.63	377.0903	8.878
64	Herbicide	Lenacil	1194	69.01	235.1453	7.779
65	Herbicide	Flufenacet	1170	70.46	386.0568	15.024
66	Insecticide	Fenazaquin	1020	69.76	307.1804	15.924
67	Herbicide	Picloram	1017	50.38	257.9623	0.484
68	Insecticide	Flucythrinate	1014	77.01	469.1933	1.233
69	Fungicide	Oxadixyl	1001	61.85	301.116	9.328
70	Pesticide	Diphenylamine	1000	69.63	170.0974	1.583
Maize 8						
1	Herbicide	Buturon	589579	56.46	254.1063	15.038
2	Herbicide	Chloroxuron	209507	54.84	308.1136	15.038
3	herbicide	Tepraloxydim	133304	64.07	359.1749	5.295
4	Insecticide	Dicrotophos Bildrin	88481	95.15	255.1094	15.038
5	Insecticide	Carbosulfan	75029	51.63	403.2008	5.595
6	Insecticide	Pyrethrin I	36223	51.94	351.1908	5.295
7	Herbicide	Fluazifop-butyl	32482	61.20	406.1201	14.189
8	Acaricide	Fenpyroximate	28819	63.36	439.2315	1.447
9	Herbicide	Neburon	27555	52.58	292.1002	11.041
10	Fungicide	Triflumizol	24925	69.19	368.0754	5.645
11	Herbicide	Sethoxydim	21704	75.89	345.223	0.947
12	Herbicide	Isoxaflutole	20517	60.25	360.0537	14.938
13	Fungicide	Pyrazophos	18824	64.90	374.095	14.189
14	Herbicide	Flazasulfuron	18369	76.03	408.0599	15.239
15	Insecticide	Pirimiphos-ethyl	18242	87.44	334.1366	8.143
16	Pesticide	Maleic hydrazide (MH)	17198	75.64	130.0615	0.698
17	Insecticide	Prothoate	17166	65.64	286.0693	15.988
18	Fungicide	Triadimenol	15547	50.75	296.1188	15.038
19	Fungicide	Propiconazole	14539	61.40	342.0793	14.988
20	Herbicide	Flurtamone	11929	58.58	351.1347	13.040
21	Fungicide	Nitrothal-isopropyl	10872	79.44	318.0951	15.988

Nr	Class	Name	Abundance	Score	m/z	RT (min)
22	Insecticide	Dinocap	8805	84.64	365.1696	0.847
23	Herbicide	Dinoseb-acetate	6953	61.45	283.0901	13.889
24	Herbicide	Dinitramine	6793	88.70	323.0956	9.342
25	Fungicide	Ofurace	6101	52.93	299.1139	9.392
26	Herbicide	Pebulate	5690	76.86	226.1243	0.648
27	Fungicide	Picoxystrobin	5535	95.00	385.1379	6.894
28	Herbicide	Pretilachlor	5284	57.45	329.1995	0.947
29	Herbicide	Methoprotryne	5053	58.56	272.1548	13.739
30	Herbicide	Lenacil	4613	77.73	257.1277	7.743
31	Pesticide	Ancymidol	4613	90.01	257.1277	7.743
32	Herbicide	Bensulide	4604	64.70	398.0655	14.189
33	Insecticide	Cevadin	4435	84.49	614.3304	5.445
34	Herbicide	Piperophos	4393	61.94	371.1624	5.145
35	Herbicide	Linuron	4279	53.20	266.0477	1.897
36	Insecticide	Lethane 384	3712	91.96	221.1323	1.447
37	Fungicide	Myclobutanil	3536	52.75	289.1191	0.798
38	Insecticide	Pyrimitate	3291	58.67	328.0815	13.040
39	Insecticide	Kadethrin	3211	87.72	414.1736	12.990
40	Herbicide	Penoxalin	3207	55.71	304.1272	3.096
41	Herbicide	Imazapyr	3191	74.94	284.1015	0.698
42	Insecticide	Jasmolin I	2992	98.14	353.2084	2.346
43	Herbicide	Oxadiazon	2976	50.61	345.0784	15.038
44	Insecticide	Mipafox	2819	63.28	200.1345	0.897
45	Herbicide	Ethalfluralin	2717	77.92	334.1001	7.294
46	Pesticide	Diphenylamine	2686	78.82	170.0963	1.597
47	Herbicide	MCPA-thioethyl	2646	71.94	245.0419	13.989
48	Insecticide	Jasmolin I	2637	82.50	353.2087	1.847
49	Fungicide	Fenamidone	2589	71.19	312.1193	7.294
50	Insecticide	Cyhalothrin	2484	66.37	472.0882	14.189
51	Insecticide	Tebupirimfos	2416	56.85	319.1253	0.798
52	Insecticide	Jasmolin I	2319	65.09	353.2078	1.447
53	Herbicide	Metribuzin	2276	65.82	232.1236	5.595
54	Insecticide	Phoxim	2274	55.93	299.06	7.094
55	Insecticide	Pirimicarb	2224	63.83	261.1335	15.339
56	Herbicide	Cinidon-ethyl	2187	60.30	411.089	0.698
57	Herbicide	EPTC	2174	65.87	207.1512	8.293
58	Insecticide	Jasmolin I	2141	91.72	353.2085	5.245
59	Herbicide	Fluoroglycofen	2025	57.87	437.035	13.889
60	Herbicide	Flamprop-isopropyl	1940	65.88	364.1105	6.095
61	Herbicide	Profluralin	1925	70.38	365.1406	2.346
62	Insecticide	Fenazaquin	1912	76.82	307.1802	13.040
63	Herbicide	Lambast	1845	51.28	324.1433	13.989
64	Pesticide	Isocarbophos	1821	57.27	290.0636	15.988
65	Insecticide	Jasmolin I	1815	68.19	353.209	3.196

Nr	Class	Name	Abundance	Score	m/z	RT (min)
66	Herbicide	Pebulate	1804	69.54	221.167	15.038
67	Herbicide	Naptalam	1686	66.49	309.1253	13.090
68	Insecticide	Deguelin	1607	72.41	412.1768	1.147
69	Herbicide	Imazamox	1578	67.43	323.1734	13.489
70	Insecticide	Chlorphoxim	1547	81.16	350.0473	8.093
71	Insecticide	Triazophos	1512	64.35	331.0996	15.888
72	Insecticide	Isofenphos	1461	52.19	363.1501	0.897
73	Insecticide	5-Hydroxyimidacloprid	1443	55.82	289.0801	15.788
74	Fungicide	Tricyclazole	1391	69.96	190.0435	9.292
75	Herbicide	Methoxyphenone	1388	59.86	241.1223	1.847
76	Insecticide	Jasmolin I	1375	81.21	353.2076	1.147
77	Herbicide	Isomethiozin	1346	57.91	269.1421	10.991
78	Herbicide	Ethiolate	1284	83.29	179.1212	4.445
79	Fungicide	Nuarimol	1257	58.93	332.0956	14.738
80	Insecticide	Bomyl	1256	72.90	305.0364	10.041
81	Fungicide	Kresoxim-methyl	1206	52.01	336.1186	12.940
82	Insecticide	Thiofanox	1200	75.20	219.1159	0.847
83	Fungicide	Cyazofamid	1191	64.93	342.0788	9.692
84	Herbicide	Florasulam	1125	50.74	382.0215	15.038
85	Insecticide	Monocrotophos	1119	73.55	241.0931	5.745
86	Fungicide	Benomyl	1115	72.14	291.1467	13.040
87	Herbicide	Triasulturon	1106	56.94	402.0619	0.598
88	Herbicide	Cloquintocet-mexyl	1106	52.92	336.1347	8.093
89	Herbicide	Prodiamine	1053	62.66	351.1276	10.192
90	Herbicide	Mefenacet	1027	50.22	321.0685	9.842
91	Insecticide	Fluenethyl	1023	80.86	259.1138	13.339
92	Herbicide	Flufenacet	1020	53.63	386.0543	14.938
93	Herbicide	Difenoxuron	1016	58.16	287.1383	10.292
94	Herbicide	Fluometuron	1011	81.28	233.0901	0.847
95	Herbicide	Cyanazine	1011	55.05	241.0932	5.745
Maize 9						
1	Herbicide	Methabenzthiazuron	917876	67.59	244.0497	13.535
2	Herbicide	Tepraloxydim	145944	61.28	359.1751	5.291
3	Pesticide	Aldicarb-sulfone (Aldoxycarb)	114746	80.91	240.0993	13.535
4	Herbicide	Trifluralin	93609	91.01	336.1159	13.036
5	Insecticide	Quintiofos	91706	71.34	330.0725	14.235
6	Herbicide	Sethoxydim	53206	78.77	350.1784	0.943
7	Insecticide	Fenpropathrin	53206	70.03	350.1784	0.943
8	Pesticide	Pyridaben	48054	53.57	365.1473	0.893
9	Fungicide	8-Hydroxychinolin	42946	78.45	168.0433	13.585
10	Acaricide	Fenpyroximate	34478	61.06	439.2313	1.393
11	Fungicide	Pyrazophos	31298	70.40	391.1199	15.934
12	Insecticide	Pyrethrin I	28112	52.35	351.1908	5.291
13	Fungicide	Dichlofluanid	19147	94.04	349.997	13.535

Nr	Class	Name	Abundance	Score	m/z	RT (min)
14	Fungicide	Isazofos	18377	67.36	331.0756	14.235
15	Insecticide	Barthrin	12867	57.79	337.1197	13.036
16	Herbicide	Fluridone	7085	72.54	352.0898	13.036
17	Insecticide	Mipafox	5165	90.87	205.0864	1.143
18	Insecticide	Cevadin	5088	90.59	614.3305	5.441
19	Insecticide	Bendiocarb	4977	54.58	241.1166	15.934
20	Insecticide	Dimetilan	3991	84.40	263.1119	0.693
21	Fungicide	Chlozolinate	3918	70.18	353.9913	13.535
22	Insecticide	Dinocap	3656	71.76	365.169	0.843
23	herbicide	Piperophos	3534	67.00	371.1618	5.141
24	Insecticide	Diafenthiuron	3500	57.69	385.2308	5.591
25	Insecticide	Jasmolin I	3125	84.49	353.2088	1.793
26	Herbicide	Buturon	2980	58.73	254.1058	15.034
27	Herbicide	Dicryl	2882	51.81	247.04	13.985
28	Herbicide	Metribuzin	2764	55.87	232.1241	5.441
29	Herbicide	Flazasulfuron	2586	68.99	408.0603	15.934
30	Insecticide	Jasmolin I	2380	97.35	353.209	2.392
31	Herbicide	Methoxyphenone	2130	78.66	241.1227	1.793
32	Fungicide	Picoxystrobin	2004	71.50	385.1397	6.890
33	Herbicide	Atraton	1996	74.90	212.1514	2.692
34	Herbicide	Lambast	1979	55.45	324.1433	13.985
35	Herbicide	Diethofencarb	1922	73.09	285.1809	15.834
36	Insecticide	Jasmolin I	1870	78.36	353.2087	5.291
37	Herbicide	Dinoseb-acetate	1849	56.47	283.0904	13.835
38	Insecticide	Azinphos-ethyl	1838	75.46	346.0458	13.585
39	Insecticide	Lethane 384	1803	92.63	221.1327	2.342
40	Herbicide	Propanil	1755	50.31	235.0396	13.835
41	Herbicide	Carbetamide	1742	67.29	259.1041	0.843
42	Pesticide	Maleic hydrazide (MH)	1683	57.18	130.0612	0.693
43	Herbicide	Naphthoxyacetic acid	1648	52.32	203.0714	0.843
44	Insecticide	Jasmolin I	1641	75.79	353.2088	3.142
45	Insecticide	Phoxim	1634	69.76	299.0605	6.990
46	Fungicide	Bupirimate	1626	62.09	317.165	15.784
47	Insecticide	Jasmolin I	1590	75.57	353.2083	1.393
48	Insecticide	MNFA	1532	81.45	235.1243	13.185
49	Herbicide	Imazamox	1517	60.07	323.1736	13.135
50	Herbicide	Naptalam	1507	75.20	309.1239	13.086
51	Insecticide	Fenazaquin	1498	61.43	307.1796	13.086
52	Herbicide	Profluralin	1491	82.45	365.1403	3.142
53	Fungicide	Propamocarb	1471	77.55	189.1583	15.934
54	Insecticide	Thanite	1443	70.05	271.1477	16.134
55	Fungicide	Benomyl	1406	79.86	291.1465	13.235
56	Herbicide	Fluorodifen	1403	53.47	346.0675	13.185
57	Fungicide	Cymoxanil (Curzate)	1369	57.60	199.0826	2.642

Nr	Class	Name	Abundance	Score	m/z	RT (min)
58	Insecticide	Phenothrin	1347	54.83	351.199	14.085
59	Fungicide	Bupirimate	1329	73.70	317.1633	13.785
60	Herbicide	Methoxyphenone	1302	72.15	263.1045	5.090
61	Herbicide	Fenaclon	1290	56.93	212.0839	8.838
62	Herbicide	Cinidon-ethyl	1262	61.80	411.0892	0.793
63	Herbicide	Quinclorac	1238	50.22	241.9755	0.494
64	Herbicide	Imazethapyr	1228	57.24	307.1788	13.185
65	Insecticide	Terbufos	1178	50.74	306.0799	9.888
66	Plant growth regulator	Azoluron	1164	66.41	253.106	13.235
67	Herbicide	Imazaquin	1148	75.70	329.1598	14.385
68	Insecticide	Aldicarb	1115	64.80	213.0675	5.541
69	Herbicide	Pretilachlor	1107	62.75	329.1982	0.893
70	Insecticide	Tebufenozide	1087	60.60	375.2025	13.135
71	Plant growth regulator	Trinexapac-ethyl	1079	81.54	253.1061	13.535
72	Insecticide	Ethoprofos	1057	61.61	260.091	15.034
73	Fungicide	Fludioxonil	1034	85.37	249.0469	13.535
74	Fungicide	Oxycarboxin	1021	78.21	290.0443	3.841
75	Insecticide	Flucythrinate	1000	66.41	469.1932	1.143
Maize 10						
1	Insecticide	Quintiofos	233682	67.98	330.073	14.238
2	Insecticide	Fenpropathrin	70069	69.16	350.1785	0.897
3	Insecticide	Carbosulfan	60104	63.28	403.2008	5.594
4	Acaricide	Fenpyroximate	39106	62.49	439.2314	1.396
5	Fungicide	Isazofos	33365	68.08	331.0758	14.238
6	Insecticide	Dicrotophos Bildrin	27386	81.21	255.1097	15.037
7	Insecticide	Pyrethrin I	25879	59.59	351.1914	5.244
8	Fungicide	Triflumizol	23998	65.58	368.0753	5.644
9	Herbicide	Flurtamone	22191	62.44	351.135	13.039
10	Insecticide	Pirimiphos-ethyl	14558	88.27	334.1367	8.092
11	Herbicide	Isoxaflutole	12436	60.90	360.0538	15.037
12	Insecticide	Dinocap	11700	91.40	365.169	0.847
13	Insecticide	Diafenthiuron	11583	57.53	407.212	5.594
14	Herbicide	Sethoxydim	10151	76.62	345.223	0.897
15	Fungicide	Methfuroxam	9742	78.78	252.0983	15.887
16	Insecticide	Fenamiphos	7591	68.32	321.1427	0.747
17	Insecticide	Bendiocarb	7149	61.63	241.1163	15.937
18	Fungicide	Pyrazophos	6214	57.69	391.1192	15.937
19	Fungicide	Propiconazole	5273	60.36	342.0799	15.037
20	Fungicide	Cyazofamid	5273	51.64	342.0799	15.037
21	Herbicide	Trifluralin	4867	76.19	336.1158	13.039
22	Herbicide	Methoprotryne	4611	82.73	272.1556	13.738
23	Insecticide	Cevadin	4305	91.98	614.3307	5.444
24	Herbicide	Piperophos	3932	77.03	371.16	5.145
25	Fungicide	Fenamidone	3579	78.14	334.1011	7.293

Nr	Class	Name	Abundance	Score	m/z	RT (min)
26	Herbicide	Penoxalin	3529	76.93	304.1271	3.245
27	Herbicide	Dinoseb-acetate	3347	66.78	283.0908	13.838
28	Insecticide	Jasmolin I	3230	77.61	353.2084	1.796
29	Insecticide	Mipafox	3147	73.08	205.0866	1.147
30	Herbicide	Dinitramine	3145	87.75	323.0951	9.291
31	Herbicide	MCPA-thioethyl	2987	71.08	245.0418	13.988
32	Herbicide	Metribuzin	2782	63.19	232.1238	5.444
33	Fungicide	Bupirimate	2720	73.70	317.1649	15.788
34	Pesticide	Maleic hydrazide (MH)	2680	59.16	130.0618	0.697
35	Insecticide	Phoxim	2670	86.63	299.0601	7.043
36	Herbicide	Imazapyr	2666	73.56	284.1019	0.747
37	Herbicide	Fenoxaprop-ethyl	2656	61.50	384.0572	14.987
38	Insecticide	Jasmolin I	2549	78.39	353.2089	5.444
39	Herbicide	Prometryn	2529	50.60	259.1725	16.037
40	Herbicide	Fenuron	2433	67.17	187.083	1.646
41	Insecticide	Pirimicarb	2348	77.95	261.1321	15.388
42	Insecticide	Prothoate	2321	53.26	286.0691	15.987
43	Insecticide	Thanite	2242	93.52	271.1485	16.137
44	Insecticide	Lethane 384	2186	85.76	221.1323	4.344
45	Herbicide	Pebulate	2060	60.59	226.1241	0.597
46	Insecticide	Phenthoate	2020	62.23	338.0662	9.341
47	Herbicide	Imazamox	1836	58.91	323.1735	13.539
48	Herbicide	Cinidon-ethyl	1760	67.06	411.0882	0.497
49	Insecticide	MNFA	1758	83.40	235.1243	13.588
50	Herbicide	Carbetamide	1713	60.37	259.1071	0.697
51	Fungicide	Bupirimate	1650	58.83	317.163	13.588
52	Insecticide	Jasmolin I	1639	74.63	353.2082	2.346
53	Insecticide	Isofenphos	1538	56.23	363.1524	0.897
54	Herbicide	Difenoxuron	1514	52.57	309.1231	13.089
55	Herbicide	Naptalam	1514	75.48	309.1231	13.089
56	Insecticide	Jasmolin I	1456	79.34	353.2089	3.195
57	Herbicide	Chlorbromuron	1391	53.76	294.9653	2.296
58	Pesticide	Thiofanox-sulfone	1330	51.71	251.1082	1.796
59	Herbicide	Flufenacet	1293	73.25	386.0567	15.037
60	Herbicide	Benoxacor	1276	67.79	282.0069	0.497
61	Insecticide	Jasmolin I	1276	83.59	353.2084	1.446
62	Herbicide	Pretilachlor	1251	56.85	329.1986	0.947
63	Insecticide	Jasmolin I	1224	80.88	353.2085	1.097
64	Herbicide	Pebulate	1209	58.51	221.1664	15.037
65	Fungicide	Binapacryl	1199	73.35	323.1255	5.394
66	Herbicide	Profluralin	1195	59.63	365.1401	3.245
67	Fungicide	Flutriafol	1175	51.13	319.1407	13.139
68	Insecticide	Flucythrinate	1163	60.94	469.194	1.247
69	Herbicide	Methoxyphenone	1116	73.06	263.1047	5.145

Nr	Class	Name	Abundance	Score	m/z	RT (min)
70	Herbicide	Ethalfluralin	1104	76.45	351.128	10.191
71	Herbicide	Prodiamine	1104	76.45	351.128	10.191
72	Insecticide	Empenthrin	1086	63.14	297.1841	15.837
73	Fungicide	Oxadixyl	1085	57.91	301.1162	7.543
74	Fungicide	Benomyl	1072	66.66	291.1428	5.694
Maize 11						
1	Herbicide	Trifluralin	129831	89.00	336.1161	13.038
2	Herbicide	Tepraloxydim	109581	61.25	359.1752	5.293
3	Insecticide	Cevadin	64639	84.47	592.3488	4.343
4	Herbicide	Sethoxydim	45425	58.88	350.1787	0.946
5	Acaricide	Fenpyroximate	28960	50.06	439.2314	1.445
6	Insecticide	Pyrethrin I	25446	55.30	351.1912	5.293
7	Herbicide	Fluridone	15955	71.56	352.0896	13.038
8	Fungicide	Methfuroxam	8267	80.62	252.099	8.041
9	Herbicide	Propazine	8267	61.88	252.099	8.041
10	Herbicide	Flamprop-isopropyl	7908	66.41	364.1103	6.992
11	Insecticide	Lethane 60	6872	50.66	308.1634	5.693
12	Insecticide	Dinocap	5865	97.14	365.1703	0.846
13	Insecticide	Diafenthiuron	5479	51.00	407.2113	5.593
14	Herbicide	Imazapyr	5277	86.66	284.1012	0.746
15	Herbicide	Dicryl	4154	58.16	247.0394	13.988
16	Insecticide	Fonofos	4154	54.40	247.0394	13.988
17	Fungicide	Picoxystrobin	3760	75.92	385.1386	6.892
18	Herbicide	Chlorbromuron	3592	53.38	294.9651	2.295
19	Herbicide	Cinidon-ethyl	3355	57.80	411.0894	0.446
20	Insecticide	Lethane 384	3054	80.70	221.1327	1.445
21	Insecticide	Mipafox	2715	74.36	205.088	1.895
22	Herbicide	Buturon	2597	52.56	254.106	15.037
23	Herbicide	Buminafos	2355	69.22	370.2506	5.643
24	Insecticide	Tebufenozide	2334	78.21	370.2503	5.593
25	Insecticide	Jasmolin I	2324	76.50	353.2099	5.443
26	Insecticide	Deguelin	2317	87.94	412.1767	1.146
27	Pesticide	Maleic hydrazide (MH)	2177	61.54	130.0611	0.696
28	Insecticide	Jasmolin I	2165	81.70	353.2076	2.395
29	Herbicide	Medinoterbacetate	2090	81.99	319.0889	13.788
30	Insecticide	Phorate	2065	73.90	278.0459	7.992
31	Fungicide	Bupirimate	2037	68.81	317.1658	6.892
32	Herbicide	Imazethapyr	1911	63.57	307.1787	13.538
33	Pesticide	Phorate-sulphoxide	1855	75.53	294.0402	13.038
34	Herbicide	Desmedipham	1848	60.49	301.1196	14.088
35	Herbicide	Orbencarb	1845	50.35	258.0707	5.893
36	Herbicide	Dinoseb-acetate	1800	93.36	283.0922	13.588
37	Pesticide	Aldicarb-sulfone (Aldoxycarb)	1799	64.66	245.0547	0.946
38	Fungicide	Fenamidone	1783	83.48	334.1009	4.993

Nr	Class	Name	Abundance	Score	m/z	RT (min)
39	Insecticide	Thiofanox	1736	77.01	241.0983	4.144
40	Herbicide	Ethofumesate	1732	50.16	309.0803	9.840
41	Fungicide	Isazofos	1615	61.37	331.0735	7.142
42	Herbicide	Imazamox	1597	61.50	323.1726	13.388
43	Fungicide	Diniconazole	1534	57.61	343.1107	11.390
44	Insecticide	Jasmolin I	1423	84.32	353.2085	1.795
45	Herbicide	Methoxyphenone	1408	71.42	241.1226	1.845
46	Insecticide	Tebufenozide	1378	67.61	370.2511	5.443
47	Insecticide	Bromophos-ethyl	1375	56.04	416.8686	0.446
48	Acaricide	Methiocarb-sulfoxide	1336	52.80	259.1134	9.191
49	Insecticide	Fluenethyl	1336	62.90	259.1134	9.191
50	Fungicide	Triamiphos	1298	74.90	295.1419	5.843
51	Herbicide	Profluralin	1273	63.78	365.1402	2.395
52	Herbicide	Oxasulfuron	1271	62.34	407.1007	5.543
53	Insecticide	Methoxyfenozide	1219	68.85	386.2461	2.745
54	Insecticide	Bendiocarb	1198	70.41	241.1171	15.937
55	Fungicide	Etaconazole	1197	59.22	345.088	13.088
56	Herbicide	Flurtamone	1150	55.04	334.1011	4.993
57	Herbicide	Sulfometuron-methyl	1122	53.29	365.0939	13.888
58	Fungicide	Dimoxystrobin	1105	69.05	349.1525	13.588
59	Fungicide	Hexaconazole	1092	50.01	331.1076	6.992
60	Fungicide	Benomyl	1084	77.34	291.1443	13.938
61	Fungicide	Binapacryl	1060	63.03	345.107	6.443
62	Herbicide	Simetryn	1053	51.96	214.1101	7.592
63	Fungicide	Thiophanate-methyl	1048	52.05	360.0791	5.593
64	Herbicide	Ethalfluralin	1047	71.22	334.1007	4.993
65	Insecticide	Flucythrinate	1044	63.45	469.1925	1.196
66	Insecticide	Demeton-O-methyl	1027	53.62	248.0536	13.288
67	Herbicide	Diuron	1009	58.61	250.0506	7.042
Maize 12						
1	Herbicide	Flazasulfuron	153778	69.78	408.0610	15.924
2	Insecticide	Quintiofos	153294	65.84	330.0731	14.274
3	Herbicide	Tepraloxydim	129215	56.17	359.1757	5.281
4	Insecticide	Phosphamidon	105532	59.67	317.1033	14.874
5	Insecticide	Carbosulfan	97922	54.38	403.2012	5.580
6	Acaricide	Fenpyroximate	53702	65.10	439.2318	1.433
7	Fungicide	Isazofos	30285	64.88	331.0764	14.274
8	Insecticide	Pyrethrin I	26077	64.99	351.1923	5.281
9	Insecticide	Dinocap	19919	79.10	365.1697	0.833
10	Herbicide	Buturon	18618	63.14	254.1065	15.024
11	Herbicide	Piperophos	12459	58.73	371.1552	20.921
12	Herbicide	Sethoxydim	10287	77.65	345.2232	0.933
13	Herbicide	Daminozide	9667	80.69	161.0919	15.974
14	Insecticide	Bendiocarb	8493	63.86	241.1181	15.924

Nr	Class	Name	Abundance	Score	m/z	RT (min)
15	Herbicide	Lambast	7240	63.64	324.1436	14.025
16	Herbicide	Propazine	6758	62.76	252.0976	15.874
17	Pesticide	Isocarbophos	6490	75.19	307.0881	13.375
18	Insecticide	Pirimicarb	6441	79.09	261.1329	15.374
19	Herbicide	Trifluralin	6258	78.81	336.1173	13.025
20	Herbicide	Dinoseb-acetate	6036	51.23	283.0913	13.175
21	Insecticide	Tebupirimfos	5992	57.82	319.1266	0.783
22	Herbicide	Cinidon-ethyl	4935	60.11	411.0888	0.483
23	Herbicide	Fluazifop-butyl	4865	60.04	406.1210	14.174
24	Herbicide	Dicryl	4225	66.80	247.0400	13.975
25	Insecticide	Jasmolin I	4211	77.79	353.2085	1.782
26	Insecticide	Isofenphos	3863	51.42	363.1536	0.883
27	Fungicide	Pyrazophos	3831	69.24	374.0956	14.174
28	Insecticide	Jasmolin I	3687	87.07	353.2088	2.382
29	Herbicide	Pretilachlor	3566	60.74	329.1993	0.933
30	Insecticide	Tebufenozide	3531	60.96	353.2213	13.275
31	Insecticide	Mipafox	3428	60.21	205.0876	1.183
32	Fungicide	Iprobenfos	3243	72.36	311.0839	1.333
33	Herbicide	Profluralin	3200	80.09	365.1405	2.382
34	Insecticide	Jasmolin I	3191	76.13	353.2075	5.281
35	Insecticide	Dinocap	2915	67.48	365.1681	13.175
36	Insecticide	Phoxim	2876	61.95	299.0602	7.029
37	Herbicide	Metribuzin	2833	66.45	232.1242	5.431
38	Fungicide	Bupirimate	2691	59.84	317.1622	15.824
39	Insecticide	Jasmolin I	2650	84.42	353.2082	1.433
40	Herbicide	EPTC	2642	60.54	190.1274	1.233
41	Insecticide	Barthrin	2586	59.07	337.1198	0.733
42	Herbicide	Naptalam	2570	75.80	309.1235	1.333
43	Insecticide	Fluenethyl	2482	75.79	281.0929	2.882
44	Insecticide	Thanite	2457	54.45	271.1488	16.124
45	Herbicide	Lenacil	2358	66.00	257.1269	13.125
46	Insecticide	Fenazaquin	2310	82.95	307.1804	13.075
47	Insecticide	Cyromazine	2291	73.85	167.1044	1.333
48	Pesticide	Diphenylamine	2290	79.97	170.0958	1.632
49	Insecticide	Bromophos-ethyl	2223	55.87	416.8679	0.433
50	Herbicide	Triasulturon	2206	59.73	402.0627	0.533
51	Herbicide	Difenoxuron	2200	64.94	287.1394	15.024
52	Insecticide	Jasmolin I	2198	59.54	353.2079	3.181
53	Fungicide	Triflumizol	2167	52.84	368.0761	5.630
54	Herbicide	Fenuron	2164	69.78	187.0862	0.633
55	Insecticide	Pirimiphos-ethyl	2150	73.79	351.1619	13.375
56	Herbicide	Trifloxysulfuron	2120	60.37	438.0709	15.874
57	Fungicide	Triamiphos	2058	78.58	295.1445	4.730
58	Insecticide	Jasmolin I	2002	64.13	353.2078	1.183

Nr	Class	Name	Abundance	Score	m/z	RT (min)
59	Insecticide	Cevadin	1983	86.86	609.3760	5.431
60	Insecticide	Flucythrinate	1971	69.09	469.1940	1.283
61	Insecticide	Formetanate	1952	71.25	239.1486	5.530
62	Fungicide	Picoxystrobin	1951	65.25	385.1395	5.780
63	Herbicide	Methoxyphenone	1842	82.53	241.1230	1.782
64	Insecticide	Deguelin	1751	65.01	412.1787	1.183
65	Herbicide	Bentazone artifact	1713	74.91	201.1000	0.783
66	Insecticide	MNFA	1702	71.03	235.1246	13.025
67	Insecticide	Dicyclanil	1644	73.59	213.0874	13.325
68	Fungicide	Dimoxystrobin	1598	64.97	349.1526	4.231
69	Fungicide	Bupirimate	1597	55.58	317.1638	13.625
70	Herbicide	Pebulate	1597	67.82	204.1422	1.632
71	Herbicide	Dinoseb	1593	55.92	241.0835	13.275
72	Acaricide	Aminocarb	1561	77.77	231.1095	0.883
73	Herbicide	Tralkoxydime	1527	64.50	352.1854	0.933
74	Fungicide	Hexythiazox	1507	58.98	375.0907	5.680
75	Fungicide	Binapacryl	1507	62.94	345.1034	14.174
76	Pesticide	Ancymidol	1491	67.52	279.1128	1.932
77	Fungicide	Nitrothal-isopropyl	1478	54.37	296.1132	1.333
78	Herbicide	Imazaquin	1448	53.94	329.1600	13.875
79	Insecticide	Methoxyfenozide	1403	75.89	391.1980	6.130
80	Herbicide	Naptalam (-H2O)	1389	75.55	291.1119	13.075
81	Herbicide	Clofop-isobutyl	1352	50.79	366.1465	5.181
82	Insecticide	Fenpropathrin	1349	62.65	367.2000	5.930
83	Insecticide	Oxamyl	1334	68.38	237.1001	6.580
84	Insecticide	Sulfirame	1310	69.63	287.0694	1.333
85	Fungicide	Fenaminosulf	1242	60.52	247.0840	3.081
86	Herbicide	Clomazone	1225	56.77	257.1061	14.474
87	Insecticide	Cyanthoate	1225	64.50	312.1125	3.181
88	Herbicide	Phenalenone	1221	76.97	203.0455	0.533
89	Fungicide	Dichlobutrazol	1159	58.89	345.1232	13.825
90	Herbicide	Cyprazine	1084	59.33	245.1278	1.582
91	Insecticide	Etrimfos	1077	52.80	293.0718	13.475
Maize 13						
1	Herbicide	Tepraloxydim	111617	60.81	359.1753	5.289
2	Herbicide	Flazasulfuron	71021	68.69	408.0607	15.932
3	Insecticide	Carbosulfan	64172	62.40	403.2008	5.589
4	Insecticide	Cevadin	56499	81.48	592.3496	4.389
5	Insecticide	Phosphamidon	51666	60.86	317.1032	14.882
6	Insecticide	Fenpropathrin	51507	69.23	350.1786	0.941
7	Insecticide	Quintiofos	44034	66.84	330.0728	14.233
8	Acaricide	Fenpyroximate	31562	64.25	439.2315	1.441
9	Insecticide	Pyrethrin I	23746	51.25	351.1917	5.289
10	Fungicide	Benalaxyl	21583	50.75	348.1534	5.139

Nr	Class	Name	Abundance	Score	m/z	RT (min)
11	Insecticide	Dinocap	11033	93.27	365.1698	0.841
12	Fungicide	Methfuroxam	10791	60.30	252.0963	15.882
13	Fungicide	Isazofos	9258	64.32	331.0766	14.233
14	Insecticide	Bendiocarb	8005	73.07	241.1169	15.932
15	Herbicide	Sethoxydim	7032	83.62	345.2229	0.941
16	Insecticide	Thanite	5899	66.38	271.1479	16.132
17	Herbicide	Propazine	5894	51.37	252.0964	15.882
18	Pesticide	Ancymidol	4865	80.66	257.1299	1.940
19	Herbicide	Prometryn	4465	64.83	259.1698	16.032
20	Pesticide	Isocarbophos	3663	56.62	307.0877	13.383
21	Herbicide	Penoxalin	3514	57.32	304.1275	3.190
22	Fungicide	Bupirimate	3247	68.29	317.1653	15.782
23	Herbicide	Naptalam (-H2O)	3032	67.20	274.0845	15.082
24	Pesticide	Maleic hydrazide (MH)	3006	70.72	130.0613	0.741
25	Herbicide	Lambast	2987	56.08	324.1435	14.033
26	insecticide	Tebufenozide	2875	63.29	353.2216	13.283
27	Insecticide	Lethane 384	2734	93.03	221.1321	1.441
28	Pesticide	Diphenylamine	2687	65.46	170.098	1.041
29	Herbicide	Trifluralin	2570	71.74	336.1167	13.034
30	Insecticide	Jasmolin I	2528	73.82	353.2084	1.840
31	Insecticide	Pyrimitate	2481	52.94	328.0841	13.034
32	Insecticide	Mipafox	2265	79.51	205.0867	1.441
33	Herbicide	Dicryl	2238	54.72	247.0398	13.983
34	Herbicide	Imazamox	2170	71.19	323.172	13.433
35	Insecticide	Fenazaquin	2115	79.76	307.1794	13.034
36	Insecticide	Methiocarb	2048	68.78	248.0721	14.882
37	Insecticide	MNFA	2037	81.54	235.1251	13.233
38	Insecticide	Jasmolin I	2020	81.50	353.2078	5.289
39	Herbicide	Imazethapyr	2018	69.07	307.1794	13.783
40	Insecticide	Tebupirimfos	1930	69.96	319.1235	0.791
41	Insecticide	Phoxim	1920	85.42	299.0599	7.038
42	Herbicide	Trifloxysulfuron	1904	53.10	438.0714	15.882
43	Herbicide	Metribuzin	1871	51.35	232.1241	5.439
44	Insecticide	Deguelin	1700	69.68	412.1768	1.141
45	Insecticide	Dicrotophos Bildrin	1686	65.69	255.1117	14.732
46	Fungicide	Bupirimate	1634	69.66	317.1638	13.583
47	Insecticide	Cartap	1586	63.17	255.0951	15.932
48	Herbicide	Pretilachlor	1558	65.19	329.1991	0.941
49	Insecticide	Jasmolin I	1477	81.58	353.2084	2.390
50	Insecticide	Tebufenozide	1451	53.57	375.2058	15.832
51	Fungicide	Nitrothal-isopropyl	1364	50.46	313.1396	15.832
52	Pesticide	Pyridaben	1359	62.51	365.1417	13.184
53	Herbicide	Profluralin	1290	70.55	365.1407	1.840
54	Insecticide	Benfuracarb	1284	72.69	433.1743	20.979

Nr	Class	Name	Abundance	Score	m/z	RT (min)
55	Herbicide	Cinidon-ethyl	1217	60.32	411.0896	0.492
56	Fungicide	Picoxystrobin	1205	69.91	385.1396	5.789
57	Insecticide	Barthrin	1202	57.40	337.1191	0.741
58	Herbicide	Acetochlor	1196	54.06	292.1034	15.132
59	Herbicide	Benoxacor	1172	62.18	282.0068	0.492
60	Herbicide	Naptalam	1144	72.89	309.1237	13.084
61	Insecticide	Methoxyfenozide	1044	55.16	391.1948	13.383
62	Insecticide	Flucythrinate	1026	63.16	469.1928	1.291
Maize 14						
1	Herbicide	Tepraloxydim	130208	58.25	359.1755	5.287
2	Herbicide	Chloroxuron	111090	56.21	308.1137	15.03
3	Insecticide	Carbosulfan	105108	52.28	403.2009	5.637
4	Insecticide	Quintiofos	78091	65.68	330.0732	14.231
5	Herbicide	Naptalam	72400	77.82	309.125	15.93
6	Herbicide	Flazasulfuron	46706	69.99	408.0607	15.93
7	Acaricide	Fenpyroximate	42637	67.05	439.2319	1.489
8	Insecticide	Pyrethrin I	30916	59.13	351.1919	5.287
9	Insecticide	Dicrotophos Bildrin	24734	89.67	255.1095	15.03
10	Fungicide	Isazofos	14767	65.6	331.0762	14.231
11	Insecticide	Dinocap	14616	81.13	365.1699	0.839
12	Fungicide	Myclobutanil	13375	56.06	289.1192	1.039
13	Herbicide	Isoxaflutole	11671	63.73	360.0539	15.03
14	Herbicide	Nitraline	9200	50.12	368.0855	15.03
15	Herbicide	Sethoxydim	8834	77.37	345.2234	0.939
16	Herbicide	MCPA-thioethyl	8151	70.24	245.0418	13.981
17	Fungicide	Methfuroxam	6495	74.12	252.0985	15.88
18	Insecticide	Cevadin	6489	85.89	614.3309	5.437
19	Insecticide	Pirimicarb	5081	88.77	261.1327	15.33
20	Insecticide	Lethane 384	4991	89	221.1328	1.889
21	Insecticide	Diafenthiuron	4936	70.09	385.2302	5.587
22	Herbicide	Dinoseb-acetate	4690	73.28	283.09	13.131
23	Pesticide	Isocarbophos	4642	67.9	307.0875	13.381
24	Herbicide	Benoxacor	4346	55.98	282.0072	0.54
25	Insecticide	Jasmolin I	4301	97.36	353.2089	1.889
26	Herbicide	Pretilachlor	4068	63.52	329.1989	0.939
27	Herbicide	Piperophos	3789	69.27	371.1621	5.137
28	Herbicide	Cinidon-ethyl	3660	61.07	411.0893	0.49
29	Herbicide	Propazine	3566	69.42	252.0986	15.88
30	Herbicide	Penoxalin	3564	56.07	304.1277	3.288
31	Insecticide	Flucythrinate	3228	65.24	469.1958	1.289
32	Insecticide	Jasmolin I	3200	85.19	353.2085	2.438
33	Acaricide	Methiocarb-sulfoxide	3193	60.34	259.109	0.69
34	Insecticide	Isofenphos	3088	59.53	363.1525	0.889
35	Insecticide	Pymetrozine	2957	72.01	218.103	0.64

Nr	Class	Name	Abundance	Score	m/z	RT (min)
36	Insecticide	Jasmolin I	2876	87.92	353.2094	5.287
37	Fungicide	Kresoxim-methyl	2744	64.04	336.1183	13.032
38	Insecticide	Pyrimitate	2713	52.24	328.0823	13.032
39	Insecticide	Tebufenozide	2696	79.86	375.2029	13.181
40	Insecticide	Deguelin	2694	67.34	412.1778	1.189
41	Herbicide	Monuron	2682	67.67	221.0444	5.237
42	Herbicide	Prometryn	2640	58.37	259.1707	16.03
43	Fungicide	Bupirimate	2546	68.84	317.1646	15.83
44	Herbicide	Metribuzin	2378	55.86	232.1241	5.437
45	Insecticide	Barthrin	2326	63.5	337.1183	0.74
46	Insecticide	Jasmolin I	2303	86.48	353.2087	3.288
47	Herbicide	Dicryl	2299	68.13	247.0396	13.981
48	Pesticide	Maleic hydrazide (MH)	2283	59.48	130.0615	0.74
49	Herbicide	Phenalenone	2279	62.71	203.0466	0.54
50	Insecticide	Jasmolin I	2276	73.83	353.2088	1.489
51	Herbicide	Profluralin	2230	75.48	365.1417	1.889
52	Insecticide	MNFA	2171	77.4	235.1254	13.431
53	Herbicide	Trifluralin	2058	77.26	336.1183	13.032
54	Herbicide	Fenoxaprop-ethyl	1995	64.63	384.0581	14.98
55	Herbicide	Imazethapyr	1844	54.89	307.1798	13.681
56	Insecticide	Jasmolin I	1818	83.74	353.2084	1.139
57	Insecticide	Tebufenozide	1738	77.51	375.2033	13.781
58	Herbicide	Imazamox	1712	52.26	323.173	13.131
59	Fungicide	Bupirimate	1523	61.48	317.1633	13.581
60	Insecticide	Cyromazine	1500	62.35	167.102	13.082
61	Plant growth regulator	Azoluron	1455	72.19	253.1067	13.431
62	Herbicide	Acetochlor	1438	52.42	287.1536	13.082
63	Herbicide	Fluazifop-butyl	1431	60.66	406.121	14.181
64	Pesticide	Pyridaben	1398	54.96	365.1436	1.489
65	Herbicide	Trifloxysulfuron	1331	55.63	438.0711	15.88
66	Fungicide	Benomyl	1278	65.79	291.1476	13.381
67	Insecticide	Fenazaquin	1222	84.83	307.1806	10.234
68	Pesticide	Diphenylamine	1218	54.05	170.0982	2.788
69	Insecticide	Thiometon	1211	59.49	247.0049	0.839
70	Insecticide	Cyanthoate	1175	74.21	312.1118	3.188
71	Fungicide	Propamocarb	1167	53.81	189.1581	15.83
72	Insecticide	Carbaryl	1147	63.59	219.1148	0.839
73	Pesticide	Aldicarb-sulfone (Aldoxycarb)	1087	58.77	245.0578	0.989
74	Herbicide	Triasulturon	1075	56.85	402.0623	0.54
75	Fungicide	Triamiphos	1055	82.67	295.1435	5.737
76	Insecticide	Methoxyfenozide	1046	72.28	391.2012	14.331
77	Insecticide	Tetram (TM)	1032	77.68	287.1551	15.88
78	Pesticide	Paraoxon-methyl	1016	73.82	265.0579	6.087
Maize 15						

Nr	Class	Name	Abundance	Score	m/z	RT (min)
1	Insecticide	Thanite	12714	83.77	271.148	16.125
2	Herbicide	Tepraloxydim	9713	61.82	359.175	5.283
3	Insecticide	Cevadin	9311	83.52	609.3753	4.383
4	Herbicide	Buturon	7025	51.73	254.1058	15.025
5	Herbicide	Cinidon-ethyl	5295	64.12	411.0886	0.486
6	Insecticide	Lethane 384	4998	75.35	204.1052	15.975
7	Fungicide	Benalaxyl	4891	68.96	348.1538	5.133
8	Insecticide	Carbosulfan	4710	53.34	403.201	5.633
9	Fungicide	Furmecyclox	3981	58.04	252.1603	14.826
10	Insecticide	Phenothrin	2809	60.49	351.1927	5.283
11	Insecticide	Pyrethrin I	2809	79.68	351.1927	5.283
12	Herbicide	Flazasulfuron	2792	70.54	408.0597	15.925
13	Fungicide	Bupirimate	2713	71.27	317.1649	15.775
14	Insecticide	MNFA	2472	74.83	235.1243	13.177
15	Herbicide	Methoprotryne	2440	74.44	272.153	16.125
16	Insecticide	Benfuracarb	2315	72.62	428.2213	5.833
17	Fungicide	Bupirimate	2082	76.98	317.1643	13.577
18	Acaricide	Fenpyroximate	1859	62.12	439.2307	1.435
19	Insecticide	Cyromazine	1639	56.93	167.1019	15.975
20	Herbicide	Sethoxydim	1596	60.34	350.1784	0.936
21	Herbicide	Ametryne	1472	56.16	228.1261	16.125
22	Herbicide	Buminafos	1469	53.01	365.2948	16.025
23	Herbicide	Fluroxypyr-meptyl	1467	53.44	367.0992	0.536
24	Insecticide	Dicrotophos Bildrin	1464	67.83	255.1112	15.075
25	Herbicide	Karbutilate	1451	55.26	297.1931	16.025
26	Insecticide	Fenazaquin	1447	70.56	307.1784	13.677
27	Fungicide	Bitertanol	1385	77.12	355.2136	16.025
28	Herbicide	Imazamox	1375	57.65	323.1739	13.427
29	Fungicide	Propamocarb	1229	81.99	189.159	15.975
30	Herbicide	Difenoxuron	1182	78.3	287.1377	15.025
31	Herbicide	Imazethapyr	1005	66.31	307.1788	13.826
Pecan 1						
1	Herbicide	Tepraloxydim	205348	61.29	359.1752	5.295
2	Insecticide	Carbosulfan	130146	53.41	403.2011	5.595
3	Insecticide	Fenpropathrin	125193	65.19	350.1786	0.948
4	Insecticide	Fenamiphos	45917	72.45	321.1385	0.748
5	Insecticide	Bendiocarb	40024	75.06	241.1166	15.938
6	Insecticide	Dinocap	31316	87.71	365.1692	0.848
7	Insecticide	Pyrethrin I	29861	51.71	351.1914	5.295
8	Herbicide	Sethoxydim	15902	71.36	345.2233	0.948
9	Fungicide	Myclobutanil	13241	51.61	289.1190	0.998
10	Insecticide	Jasmolin I	5910	72.02	353.2087	1.847
11	Insecticide	Mipafox	5369	87.90	205.0862	1.447
12	Insecticide	Thanite	5198	95.63	271.1477	16.138

Nr	Class	Name	Abundance	Score	m/z	RT (min)
13	Insecticide	Phosphamidon	5117	67.95	317.1031	14.889
14	Herbicide	Pretilachlor	5057	56.67	329.1987	0.948
15	Herbicide	Cinidon-ethyl	5046	58.73	411.0887	0.498
16	Insecticide	Jasmolin I	4488	92.84	353.2091	2.397
17	Herbicide	Ethalfluralin	4417	75.43	334.0998	15.888
18	Insecticide	Jasmolin I	4274	89.34	353.2082	1.447
19	Insecticide	Jasmolin I	3774	95.55	353.2087	5.445
20	Herbicide	Metribuzin	3751	78.38	232.1238	5.445
21	Insecticide	Isofenphos	3421	54.73	363.1533	0.898
22	Herbicide	Penoxalin	3391	60.27	304.1272	3.296
23	Herbicide	Naptalam	3318	75.30	309.1245	13.090
24	Acaricide	Methiocarb-sulfoxide	3096	58.75	259.1088	0.698
25	Insecticide	Pymetrozine	3029	57.05	218.1028	0.648
26	Fungicide	Fenamidone	3017	53.78	334.1000	15.888
27	Insecticide	Lethane 384	2947	87.86	221.1330	4.395
28	Insecticide	Jasmolin I	2750	74.87	353.2087	1.147
29	Insecticide	Jasmolin I	2723	81.05	353.2085	3.196
30	Herbicide	Pyriftalid	2530	64.86	319.0727	15.938
31	Acaricide	Methiocarb-sulfoxide	2350	52.17	259.1119	13.340
32	Fungicide	Bupirimate	2342	67.83	317.1654	15.788
33	Insecticide	Cyanthoate	2338	86.07	295.0875	10.442
34	Insecticide	Flucythrinate	2029	80.58	469.1937	1.347
35	Insecticide	MNFA	1907	70.26	235.1249	13.589
36	Fungicide	Thiophanate-methyl	1787	57.51	365.0366	13.040
37	Fungicide	Bupirimate	1760	73.69	317.1634	13.589
38	Fungicide	Penconazole	1711	61.76	301.0988	14.139
39	Herbicide	Daminozide	1685	76.42	183.0738	1.847
40	Insecticide	Cevadin	1678	89.41	609.3754	5.445
41	Insecticide	Kadethrin	1665	69.09	414.1720	13.040
42	Herbicide	Imazamox	1628	62.36	323.1747	13.440
43	Insecticide	4-Nitrophenol	1604	54.69	157.0610	15.988
44	Insecticide	Tebufenozide	1541	62.52	353.2200	13.240
45	Insecticide	Fenazaquin	1537	67.89	307.1786	13.739
46	Fungicide	Benomyl	1521	55.69	291.1479	13.190
47	Herbicide	Benoxacor	1521	57.79	282.0065	0.598
48	Herbicide	Profluralin	1436	70.16	365.1395	3.296
49	Insecticide	Fluenethyl	1383	51.46	259.1144	13.340
50	Fungicide	Dimoxystrobin	1367	72.42	327.1685	13.040
51	Insecticide	Jasmolin I	1366	59.35	353.2086	4.395
52	Herbicide	Dinoseb	1361	83.27	241.0811	10.492
53	Herbicide	Triasulturon	1357	57.62	402.0630	0.548
54	Pesticide	Ancymidol	1332	63.88	257.1290	1.947
55	Insecticide	Etrimfos	1235	52.83	293.0726	9.942
56	Insecticide	Spinosyn D	1183	74.04	763.5134	18.587

Nr	Class	Name	Abundance	Score	m/z	RT (min)
57	Herbicide	Prodiamine	1165	69.36	368.1526	0.948
58	Herbicide	Isoxaben	1159	64.68	355.1653	14.189
59	Fungicide	Binapacryl	1154	65.80	323.1240	0.798
60	Herbicide	Prometryn	1123	52.18	242.1412	14.039
61	Herbicide	Simazine	1071	61.73	219.1132	0.648
62	Insecticide	Paraoxon	1065	72.90	293.0876	9.792
63	Herbicide	Imazapyr	1058	65.80	284.1015	0.748
Pecan 2						
1	Insecticide	Phosphamidon	278397	61.84	317.1033	14.877
2	Insecticide	Cevadin	117181	82.05	592.3492	4.383
3	Herbicide	Tepraloxydim	116251	61.45	359.1751	5.283
4	Insecticide	Carbosulfan	93823	52.20	403.2009	5.633
5	Insecticide	Fenpropathrin	89045	64.60	350.1786	0.936
6	Acaricide	Fenpyroximate	50169	66.45	439.2318	1.435
7	Herbicide	Cinidon-ethyl	38728	60.91	411.0892	0.486
8	Insecticide	Pyrethrin I	25060	54.13	351.1913	5.283
9	Fungicide	Benalaxyl	21837	54.33	348.1538	5.133
10	Herbicide	Dinoseb-acetate	21724	70.29	283.0901	13.178
11	Herbicide	Phenalenone	20557	75.10	203.0474	0.486
12	Fungicide	Thiophanate-methyl	17297	86.73	365.0339	13.078
13	Insecticide	Dinocap	16474	93.67	365.1699	0.836
14	Pesticide	Isocarbophos	16060	72.39	307.0875	13.428
15	insecticide	Diafenthiuron	15112	64.71	407.2117	5.633
16	Insecticide	Sethoxydim	12313	79.29	345.2228	0.936
17	Insecticide	Methiocarb	11859	72.51	248.0717	14.927
18	Herbicide	MCPA-thioethyl	10111	81.56	245.0415	13.978
19	Insecticide	Lethane 384	8403	89.22	221.1332	5.433
20	Insecticide	Bendiocarb	7910	74.61	241.1165	15.927
21	Herbicide	Propazine	7410	67.23	252.0962	15.877
22	Herbicide	Flazasulfuron	6933	66.58	408.0604	15.927
23	Insecticide	Etrimfos	6612	90.03	293.0721	7.082
24	Herbicide	Daminozide	6311	80.87	161.0919	15.977
25	Pesticide	Ancymidol	5523	83.83	257.1300	1.935
26	Insecticide	Terbufos	5237	62.30	289.0529	2.085
27	Herbicide	Pretilachlor	5190	69.99	329.1994	0.936
28	Insecticide	Jasmolin I	5038	82.54	353.2090	1.835
29	Insecticide	Pyrimitate	4583	65.66	328.0823	14.028
30	Herbicide	Piperophos	4220	72.35	371.1616	5.283
31	Herbicide	Penoxalin	4025	56.21	304.1275	3.234
32	Insecticide	p,p'-Dichlorophenylacetate methyl ester	3854	55.34	312.0561	13.078
33	Herbicide	Lambast	3682	52.05	324.1427	14.028
34	Insecticide	Jasmolin I	3563	87.92	353.2098	2.385
35	Herbicide	Dicryl	3456	57.90	247.0396	13.978

Nr	Class	Name	Abundance	Score	m/z	RT (min)
36	Acaricide	Dinobuton	3258	72.88	349.1025	0.536
37	Insecticide	Jasmolin I	3073	92.12	353.2088	5.283
38	Fungicide	Penconazole	3061	54.77	301.0998	14.128
39	Insecticide	Mipafox	3014	57.87	205.0872	1.435
40	Insecticide	Isofenphos	2982	53.10	363.1535	0.936
41	Herbicide	Bentranil	2808	64.17	241.0989	4.183
42	Herbicide	Benoxacor	2416	51.63	282.0073	0.536
43	Pesticide	Phorate-sulphoxide	2399	55.00	277.0124	13.578
44	Fungicide	Fuberidazole	2345	74.48	185.0717	14.877
45	Insecticide	Thiofanox	2281	63.37	241.0988	4.183
46	Herbicide	Imazamox	2157	75.12	323.1724	13.428
47	Insecticide	Jasmolin I	2033	81.86	353.2080	1.435
48	Plant growth regulator	Azoluron	1982	72.51	231.1244	15.877
49	Insecticide	Dinocap	1929	71.48	365.1736	13.178
50	Herbicide	Barban	1919	53.06	258.0059	1.285
51	Herbicide	Imazethapyr	1907	57.48	307.1789	13.228
52	Herbicide	Difenoxuron	1889	59.29	309.1235	13.128
53	Herbicide	Naptalam	1889	70.51	309.1235	13.128
54	Herbicide	Buturon	1848	61.21	254.1063	15.077
55	Insecticide	Tebupirimfos	1840	52.55	319.1265	0.786
56	Fungicide	Bupirimate	1759	65.52	317.1639	15.827
57	Herbicide	Clofop-isobutyl	1751	60.96	371.1041	10.430
58	Fungicide	Picoxystrobin	1654	72.11	385.1374	5.783
59	Insecticide	Bromophos-ethyl	1639	57.13	416.8683	0.436
60	Herbicide	Chlorbromuron	1604	55.83	294.9654	2.934
61	Insecticide	Fenazaquin	1574	79.86	307.1791	13.728
62	Insecticide	Jasmolin I	1570	72.53	353.2087	3.184
63	Fungicide	Bupirimate	1475	74.12	317.1636	13.628
64	Fungicide	Propamocarb	1459	77.61	189.1586	15.927
65	Insecticide	Phoxim	1431	54.46	316.0887	13.578
66	Insecticide	Cyanthoate	1369	59.74	312.1120	3.234
67	Insecticide	Tebufenozide	1364	83.12	375.2042	13.278
68	Herbicide	Prodiamine	1332	54.01	368.1525	0.936
69	Insecticide	Bomyl	1314	54.18	283.0537	12.029
70	Herbicide	Picolinafen	1307	67.01	377.0935	0.636
71	Herbicide	Diethofencarb	1290	56.50	285.1776	13.428
72	Insecticide	Phosalone	1288	83.64	367.9931	14.327
73	Herbicide	Imazapyr	1287	74.26	279.1472	13.728
74	Herbicide	Jasmolin I	1272	96.60	353.2088	4.433
75	Herbicide	Imazamox	1258	68.24	323.1703	14.627
76	Insecticide	Empenthrin	1190	72.46	297.1853	15.877
77	Insecticide	Thanite	1167	69.53	271.1475	16.127
78	Herbicide	Molinate	1128	53.16	188.1095	0.736
79	Fungicide	Triamiphos	1117	83.63	295.1432	6.233

Nr	Class	Name	Abundance	Score	m/z	RT (min)
80	Herbicide	Fluazifop-butyl	1102	51.77	401.1643	5.483
81	Insecticide	Tetram (TM)	1064	70.00	287.1561	15.877
82	Herbicide	Methoxyphenone	1051	70.96	263.1040	1.835
Pecan 3						
1	Herbicide	Tepraloxydim	175878	57.35	359.1756	5.245
2	Insecticide	Fenpropathrin	84292	67.63	350.1785	0.948
3	Herbicide	Metazachlor	80507	57.97	295.1332	13.440
4	Acaricide	Fenpyroximate	40682	62.75	439.2315	1.447
5	Insecticide	Pyrethrin I	32261	56.21	351.1916	5.245
6	Insecticide	Bendiocarb	30201	75.80	241.1168	15.938
7	Insecticide	Diafenthiuron	15219	58.17	407.212	5.595
8	Herbicide	Sethoxydim	13319	78.95	345.2231	0.948
9	Insecticide	Dinocap	12948	82.31	365.1696	0.848
10	Herbicide	Flazasulfuron	10594	73.62	408.0596	15.938
11	Insecticide	Thanite	9104	95.93	271.1475	16.138
12	Insecticide	Lethane 384	7553	86.27	221.1332	5.245
13	Insecticide	Mipafox	7197	86.80	205.0865	1.148
14	Insecticide	Quintiofos	5572	68.45	330.0728	14.239
15	Herbicide	Piperophos	5009	76.13	371.1608	5.245
16	Insecticide	Jasmolin I	3809	89.84	353.2092	2.347
17	Herbicide	Pretilachlor	3638	56.41	329.1984	0.948
18	Herbicide	Imazapyr	3607	73.63	284.1022	0.698
19	Fungicide	Bupirimate	3450	87.21	317.1647	15.788
20	Insecticide	Jasmolin I	3394	98.30	353.2083	1.847
21	Insecticide	Fenamiphos	3344	55.46	321.1404	0.898
22	Herbicide	Penoxalin	3253	59.19	304.1274	3.046
23	Insecticide	Tebupirimfos	3077	62.28	319.1256	0.798
24	Herbicide	Daminozide	3025	86.17	183.0738	1.397
25	Herbicide	Naptalam	2833	79.40	309.1243	13.090
26	Herbicide	Metribuzin	2806	62.51	232.1245	5.595
27	Acaricide	Methiocarb-sulfoxide	2777	61.96	259.109	0.698
28	Fungicide	Binapacryl	2737	61.56	323.1228	0.798
29	Insecticide	Jasmolin I	2693	98.32	353.2085	5.095
30	Fungicide	Furmecyclox	2639	61.06	252.1611	14.838
31	Fungicide	Bupirimate	2412	64.41	317.1645	13.589
32	Insecticide	Jasmolin I	2411	99.11	353.2087	1.447
33	Herbicide	Trifluralin	2341	82.30	336.1166	13.040
34	Insecticide	Jasmolin I	2265	77.25	353.2094	1.148
35	Herbicide	Benoxacor	2247	52.78	282.0061	0.498
36	Herbicide	Deiquat	2176	75.00	207.0898	13.440
37	Insecticide	p,p'-Dichlorophenylacetate methyl ester	1872	53.84	312.0557	7.544
38	Insecticide	MNFA	1856	70.83	235.1242	13.340
39	Insecticide	Cevadin	1757	92.27	609.3754	5.245

Nr	Class	Name	Abundance	Score	m/z	RT (min)
40	Insecticide	Flucythrinate	1719	69.73	469.1949	1.198
41	Insecticide	Prothoate	1707	53.94	303.0981	0.998
42	Fungicide	Picoxystrobin	1678	73.25	385.1393	13.090
43	Herbicide	Imazamox	1668	58.70	323.1748	13.440
44	Insecticide	Cyromazine	1570	65.06	167.102	15.888
45	Herbicide	Methoxyphenone	1550	72.87	241.1219	1.847
46	Insecticide	Jasmolin I	1476	79.16	353.2091	3.146
47	Insecticide	Barthrin	1471	54.06	337.1187	0.748
48	Herbicide	Tralkoxydime	1429	66.06	352.1874	0.948
49	Herbicide	Profluralin	1291	79.19	365.1414	3.096
50	Insecticide	Fenazaquin	1258	74.37	307.18	13.739
51	Fungicide	N,N-Dimethyl-N'-p-tolylsulfonyldiamide (DMST)	1182	50.42	215.0833	0.648
52	Fungicide	Isazofos	1137	65.46	331.076	14.239
53	Insecticide	Pirimiphos-ethyl	1072	67.42	351.1633	13.340
54	Herbicide	Karbutilate	1054	70.39	297.1933	16.038
Pecan 4						
1	Insecticide	Quintiofos	516566	64.97	330.0733	14.282
2	Herbicide	Tepraloxydim	150377	59.81	359.1753	5.289
3	Herbicide	Dinoseb-acetate	105661	78.54	283.0900	13.133
4	Pesticide	Isocarbophos	94580	73.27	307.0872	13.383
5	Insecticide	Carbosulfan	92264	53.36	403.2010	5.638
6	Fungicide	Isazofos	91008	67.69	331.0760	14.282
7	Insecticide	Phosphamidon	44692	61.62	317.1030	14.882
8	Insecticide	Pyrethrin I	39303	58.15	351.1915	5.289
9	Acaricide	Fenpyroximate	36736	64.45	439.2315	1.440
10	Herbicide	Sethoxydim	23396	68.93	328.1972	0.941
11	Herbicide	Trifloxysulfuron	20807	73.57	438.0702	15.881
12	Herbicide	Flazasulfuron	15895	75.19	408.0601	15.282
13	Insecticide	Pyrimitate	15507	65.31	328.0819	13.033
14	Insecticide	Diafenthiuron	14283	65.48	407.2118	5.638
15	Insecticide	Dinocap	13487	83.54	365.1695	0.841
16	Herbicide	Fluazifop-butyl	10792	76.38	406.1210	14.182
17	Insecticide	Bendiocarb	9076	79.70	241.1176	15.931
18	Fungicide	Myclobutanil	8288	54.60	289.1192	0.991
19	Fungicide	Pyrazophos	6425	62.90	374.0954	14.182
20	Insecticide	Cevadin	5610	85.22	614.3310	5.289
21	Herbicide	Piperophos	5320	53.50	371.1625	5.289
22	Insecticide	Pyrimitate	5320	58.55	328.0827	16.481
23	Insecticide	Thanite	4815	87.72	271.1484	16.131
24	Insecticide	Mipafox	4663	76.68	205.0861	1.141
25	Insecticide	Lethane 384	4298	81.84	221.1331	1.490
26	Insecticide	Fenthion	4017	57.63	296.0515	15.981
27	Herbicide	Mefenacet	3871	67.16	299.0866	16.031

Nr	Class	Name	Abundance	Score	m/z	RT (min)
28	Insecticide	Tebupirimfos	3788	51.39	319.1252	0.791
29	Insecticide	Jasmolin I	3591	93.91	353.2083	1.890
30	Herbicide	Cinidon-ethyl	3538	59.55	411.0884	0.491
31	Insecticide	Jasmolin I	3417	85.96	353.2091	2.440
32	Insecticide	Etrimfos	3416	70.85	293.0724	7.037
33	Fungicide	Propiconazole	3392	53.77	359.1047	15.582
34	Herbicide	Pretilachlor	3202	53.00	329.1989	0.941
35	Herbicide	Metribuzin	2634	59.02	232.1238	5.438
36	Insecticide	Deguelin	2591	71.78	412.1774	1.141
37	Insecticide	Barthrin	2542	61.79	337.1190	0.741
38	Acaricide	Methiocarb-sulfoxide	2479	63.43	259.1090	0.691
39	Herbicide	Pyridate	2426	64.49	401.1090	16.081
40	Insecticide	Jasmolin I	2371	73.96	353.2089	1.490
41	Insecticide	Jasmolin I	2321	89.06	353.2086	5.438
42	Herbicide	Methoxyphenone	2210	77.78	241.1230	1.840
43	Herbicide	Bensulfuron-methyl	2177	91.77	411.0956	14.382
44	Fungicide	Binapacryl	2048	78.67	323.1246	0.791
45	Herbicide	Imazamox	2012	75.86	323.1742	13.483
46	Insecticide	MNFA	1990	59.88	235.1250	13.183
47	Fungicide	Bupirimate	1865	60.15	317.1656	15.782
48	Insecticide	Bromophos-ethyl	1843	54.49	416.8691	0.441
49	Insecticide	Flucythrinate	1816	72.73	469.1946	1.291
50	Insecticide	Nitenpyram	1711	74.40	271.0933	15.831
51	Insecticide	Tebufenozide	1686	79.79	375.2036	13.283
52	Herbicide	Monuron	1672	59.39	221.0438	5.239
53	Fungicide	Propamocarb	1653	73.75	189.1585	16.031
54	Herbicide	MCPA-thioethyl	1561	78.98	245.0416	13.982
55	Herbicide	Medinoterbacetate	1497	65.84	297.1077	15.931
56	Herbicide	Difenoxuron	1468	60.81	309.1238	13.133
57	Insecticide	Coumaphos	1453	78.20	380.0471	13.683
58	Fungicide	Bupirimate	1439	64.35	317.1634	13.583
59	Herbicide	Furilazole	1425	70.13	278.0355	2.140
60	Herbicide	Naptalam	1408	68.36	309.1243	13.133
61	Fungicide	Thiophanate-methyl	1406	73.86	365.0344	13.083
62	Plant growth regulator	Dikegulac	1372	84.73	275.1125	7.337
63	Herbicide	Imazamox	1357	64.89	323.1734	14.732
64	Insecticide	Jasmolin I	1331	66.10	353.2076	1.191
65	Insecticide	Fenazaquin	1289	83.17	307.1801	14.832
66	Insecticide	Isofenphos	1186	61.20	363.1506	15.532
67	Insecticide	Cartap	1178	69.01	255.0958	8.736
68	Insecticide	Demeton-S-methylsulfone	1074	72.24	284.9993	0.541
69	Herbicide	Profluralin	1054	71.31	365.1403	3.239
70	Insecticide	Methamidophos	1040	54.45	142.0097	14.232
71	Fungicide	Triamiphos	1030	74.02	295.1446	6.138

Table S14 Agrochemicals detected in maize field and pecan orchard soil samples following chemical screening (negative ionisation). Arranged according to their abundance.

Nr	Class	Name	Abundance	Score	m/z	RT (min)
Maize 1						
1	Herbicide	Hexazinone	146815	81.24	311.1703	6.945
2	Insecticide	Cevadin	61343	59.93	636.3339	3.247
3	Herbicide	Medinoterbacetate	30286	80.70	341.0963	0.649
4	Herbicide	Dinoseb-acetate	27288	80.41	341.0963	0.649
5	Fungicide	Flusilazol	26777	77.13	314.0945	3.847
6	Herbicide	Diflufenican	26437	82.01	439.0704	0.599
7	Insecticide	Isolan	12903	87.03	210.1266	3.097
8	Insecticide	Etrimfos	9442	50.67	291.0544	6.995
9	Acaricide	Aminocarb	6852	92.68	267.1364	7.944
10	Insecticide	Tebufenozide	5957	82.05	397.2131	7.445
11	Herbicide	Diethofencarb	5573	58.04	266.1403	7.345
12	Insecticide	Fenazaquin	5268	81.26	351.1725	7.345
13	Insecticide	Bomyl	4347	53.52	327.0505	4.846
14	Pesticide	2,4-Dinitrophenol	3970	58.05	183.0036	7.045
15	herbicide	Simetryn	3671	60.45	212.0986	6.545
16	Herbicide	Cyprazine	3149	57.94	286.1082	7.345
17	Acaricide	Aminocarb	2897	76.12	267.1363	7.045
18	Pesticide	4,4'-Bipyridyl	2675	75.42	215.0811	3.097
19	Herbicide	Desmedipham	2668	71.33	359.1244	7.095
20	Insecticide	Allethrin II	1834	69.71	391.1736	6.945
21	Plant growth regulator	Trinexapac-ethyl	1647	56.67	251.0949	3.597
22	Herbicide	Atrazine-desethyl-desisopropyl	1552	59.52	204.0294	3.247
23	Insecticide	Dinocap	1539	66.03	423.1776	6.995
24	Herbicide	Difenoxuron	1533	55.41	285.1210	3.397
25	Herbicide	Fluometuron	1487	66.72	231.0745	3.147
26	Herbicide	Cyanazine	1454	55.39	239.0804	3.147
27	Herbicide	Aziprotryne	1409	60.00	270.0775	5.346
28	Herbicide	Chloral methyl hemiacetal	1291	66.51	238.9465	6.895
29	Fungicide	Myclobutanil	1196	50.23	287.1079	7.395
30	Herbicide	Oxadiargyl	1154	51.93	385.0371	6.445
31	Plant growth regulator	Dikegulac	1140	82.76	333.1212	8.044
32	Insecticide	Tinox	1129	64.27	275.0181	6.845
33	Insecticide	Demeton-O-methyl	1129	64.27	275.0181	6.845
34	Herbicide	Erbon	1105	56.04	424.9089	7.145
35	Insecticide	Tetrachlorvinphos	1105	52.92	424.9089	7.145
36	Herbicide	Deiquat	1092	76.08	183.0936	3.497
37	Fungicide	Bupirimate	1030	55.82	361.1539	3.647
38	Herbicide	Metribuzin	1023	71.94	273.1027	4.796
Maize 2						

Nr	Class	Name	Abundance	Score	m/z	RT (min)
1	Fungicide	Flusilazol	376702	72.51	314.0954	3.838
2	Insecticide	Cevadin	101587	59.41	636.3341	3.238
3	Herbicide	Medinoterbacetate	16062	78.64	341.0962	0.491
4	Insecticide	Demeton-O	11998	87.50	257.0444	3.138
5	Herbicide	Diethofencarb	9031	74.38	266.1404	7.286
6	Pesticide	2,4-Dinitrophenol	8182	62.54	183.0036	7.836
7	Insecticide	Tebufenozide	7741	78.47	397.2128	7.486
8	Fungicide	Furmecyclox	7494	54.30	250.1444	7.036
9	Herbicide	Florasulam	7183	59.78	404.0261	7.186
10	Insecticide	Disulfoton	6076	59.26	273.0226	1.840
11	Herbicide	Cyprazine	5267	59.98	286.1082	7.386
12	Insecticide	Fenazaquin	5147	80.04	365.1868	6.986
13	Pesticide	4,4'-Bipyridyl	4017	82.51	215.0828	3.138
14	Herbicide	Diethofencarb	3920	59.48	266.1403	4.038
15	Acaricide	Aminocarb	3416	93.68	267.1360	7.336
16	Fungicide	2,3-Dichlorophenol	3090	54.62	206.9632	8.135
17	Acaricide	Aminocarb	2147	61.83	267.1370	4.388
18	Fungicide	Quassin	2077	63.17	389.1980	6.836
19	Fungicide	2,3-Dichlorophenol	2010	50.71	206.9636	5.388
20	Fungicide	Benomyl	1884	81.45	349.1524	6.936
21	Herbicide	Dinoseb-acetate	1773	67.84	341.0988	7.886
22	Herbicide	Glyphosate	1669	64.58	228.0296	3.088
23	Fungicide	2,3-Dichlorophenol	1662	55.29	206.9633	3.488
24	Insecticide	Triflumuron	1656	51.80	357.0282	7.186
25	Herbicide	Bentazone	1575	58.32	239.0493	7.336
26	Fungicide	2-Phenylphenol	1536	71.17	169.0647	0.890
27	Herbicide	Ethiolate	1447	70.95	220.1014	7.086
28	Insecticide	Diafenthiuron	1421	65.64	383.2156	7.186
29	Herbicide	Isoxaben	1307	52.70	391.1868	6.836
30	Herbicide	Cyanazine	1297	62.74	239.0826	3.088
31	Herbicide	Picloram	1292	68.46	238.9176	7.786
32	Fungicide	Cymoxanil (Curzate)	1259	78.58	257.0899	3.138
33	Herbicide	EPTC	1198	53.59	234.1149	7.186
34	Fungicide	Iprobenfos	1137	63.14	347.1106	3.088
35	Fungicide	Triamiphos	1123	79.99	293.1293	6.836
36	Herbicide	Atrazine-desethyl-desisopropyl	1091	51.49	204.0307	3.288
37	Herbicide	Morfamquate	1072	61.76	513.2673	6.986
38	Insecticide	Dimetilan	1034	80.50	285.1204	3.438
39	Herbicide	Deiquat	1007	82.38	183.0936	3.238
40	Insecticide	Etrimfos	1000	58.17	291.0568	6.836
Maize 3						
1	Insecticide	Cevadin	154931	59.58	636.3342	3.234
2	Insecticide	Tebufenozide	23387	81.68	351.2075	6.982
3	Pesticide	2,4-Dinitrophenol	19772	72.92	183.0036	7.032

Nr	Class	Name	Abundance	Score	m/z	RT (min)
4	Herbicide	Dinoseb-acetate	18147	80.01	341.0964	0.486
5	Herbicide	3,4-Dichloraniline	16051	56.54	159.9719	0.486
6	Fungicide	Furmecyclox	8559	59.82	250.1452	7.082
7	Insecticide	Tebufenozide	8333	81.55	397.2133	7.481
8	Herbicide	Cyprazine	6106	61.56	286.1088	7.381
9	Insecticide	Carbosulfan	5757	58.84	379.2044	7.032
10	Insecticide	DNOC (2,4-Dinitro-o-kresol)	5627	65.07	257.0408	3.084
11	Herbicide	Diethofencarb	5302	56.38	266.1403	4.683
12	Acaricide	Aminocarb	5065	70.46	267.1362	7.331
13	Insecticide	Isolan	4734	66.90	210.1264	3.134
14	Insecticide	Disulfoton	4099	73.09	273.0225	1.885
15	Pesticide	Ancymidol	4030	65.21	255.1129	6.882
16	Acaricide	Aminocarb	3794	89.48	267.1354	7.831
17	Herbicide	Mefenacet	3595	62.41	297.0701	3.534
18	Fungicide	2,3-Dichlorophenol	3186	56.57	206.9629	8.131
19	Pesticide	4,4'-Bipyridyl	2788	81.64	215.0826	3.134
20	Herbicide	Falone	2562	70.23	708.9284	7.281
21	Fungicide	Myclobutanil	2488	55.36	287.1059	7.381
22	Herbicide	Bentazone	2398	64.84	239.0510	7.331
23	Acaricide	Aminocarb	2287	84.75	267.1369	4.883
24	Herbicide	Medinoterbacetate	2155	82.59	341.0983	3.134
25	Pesticide	Butocarboxim-sulphoxide	1660	62.41	265.0866	3.134
26	Herbicide	Monolinuron	1556	55.55	213.0456	6.182
27	Insecticide	Flufenoxuron	1516	62.11	533.0351	5.583
28	Insecticide	Tebupirimfos	1372	57.09	363.1156	7.331
29	Fungicide	Bitertanol	1318	65.69	382.1796	7.181
30	Herbicide	Fluometuron	1230	77.53	231.0762	3.084
31	Fungicide	Ethylendibromide	1119	55.66	232.8653	8.331
32	Herbicide	Aziprotryne	1093	60.72	270.0782	5.333
Maize 4						
1	Insecticide	Cevadin	96898	51.49	636.3335	3.236
2	Herbicide	Medinoterbacetate	27502	80.53	341.0963	0.738
3	Herbicide	Dinoseb-acetate	25995	80.45	341.0963	0.738
4	Herbicide	Chloroxuron	22682	58.28	335.0799	6.934
5	Acaricide	Aminocarb	13688	91.60	267.1363	7.933
6	Insecticide	Isolan	9399	84.06	210.1264	3.086
7	Insecticide	Disulfoton	9268	71.08	273.0231	1.837
8	Pesticide	2,4-Dinitrophenol	8131	73.70	183.0034	7.033
9	Insecticide	Demeton-O	7548	63.82	257.0421	3.136
10	Insecticide	Tebufenozide	7344	83.22	351.2071	6.983
11	Insecticide	DNOC (2,4-Dinitro-o-kresol)	6690	80.35	257.0422	3.086
12	Fungicide	Furmecyclox	6060	55.92	250.1446	7.033
13	Insecticide	Tebufenozide	6006	88.93	397.2119	7.433

Nr	Class	Name	Abundance	Score	m/z	RT (min)
14	Herbicide	Diethofencarb	5342	57.31	266.1402	7.283
15	Pesticide	4,4'-Bipyridyl	3834	80.96	215.0826	3.136
16	Fungicide	2,3-Dichlorophenol	3682	51.42	206.9635	8.133
17	Insecticide	Fenazaquin	3550	80.16	351.1723	6.934
18	Fungicide	Triamiphos	2933	70.15	293.1298	6.834
19	Herbicide	Simetryn	2633	57.96	212.0981	6.534
20	Insecticide	Pirimicarb	2476	67.10	237.1378	6.934
21	Herbicide	Cyprazine	2327	56.45	286.1083	7.333
22	Acaricide	Aminocarb	2263	75.06	267.1367	7.033
23	Fungicide	2,3-Dichlorophenol	2181	59.80	206.9638	0.238
24	Herbicide	Dinoseb	1980	56.89	299.0893	3.386
25	Insecticide	Benzoximate	1833	63.85	408.0842	4.984
26	Herbicide	Metoxuron	1792	55.17	287.0785	3.286
27	Fungicide	Hexythiazox	1744	53.64	351.0935	3.336
28	Insecticide	Prothoate	1721	55.41	330.0588	5.034
29	Insecticide	Thiofanox	1575	54.17	217.0993	3.286
30	Insecticide	5-Hydroxyimidacloprid	1530	57.81	330.0588	5.034
31	Fungicide	Cymoxanil (Curzate)	1504	74.21	257.0896	3.136
32	Herbicide	Defenurone	1486	68.47	195.0777	6.934
33	Insecticide	Barthrin	1479	54.62	335.1058	3.286
34	Herbicide	Picolinafen	1427	70.22	375.0758	7.283
35	Fungicide	Trifloxystrobin	1424	66.32	407.1236	3.336
36	Fungicide	Benomyl	1398	58.68	349.1539	3.536
37	Fungicide	Etridiazole	1380	57.74	306.9290	0.788
38	Herbicide	Mefenacet	1359	51.96	357.0939	3.086
39	Fungicide	Diniconazole	1346	55.69	324.0690	6.884
40	Herbicide	Chloridazone	1345	60.04	220.0283	3.386
41	Fungicide	Benomyl	1284	57.26	349.1531	6.884
42	Fungicide	Etaconazole	1271	51.65	386.0698	3.835
43	Insecticide	Clenpirin	1237	50.49	343.0975	3.486
44	Insecticide	Gossypol	1233	65.05	577.2099	3.036
45	Fungicide	Oxadixyl	1199	74.32	277.1198	3.286
46	Insecticide	Dimetilan	1197	73.12	285.1204	3.336
47	Herbicide	Naphthoxyacetic acid	1178	64.33	261.0765	3.086
48	Herbicide	Cyanazine	1159	58.85	239.0812	3.286
49	Insecticide	1-Naphthol	1156	67.15	189.0543	3.336
50	Herbicide	Desmedipham	1123	76.64	359.1259	3.136
51	Fungicide	Cyprodinil	1107	75.62	270.1237	7.883
52	Insecticide	Nitenpyram	1090	54.22	315.0882	3.785
53	Fungicide	Propiconazole	1089	51.65	386.0698	3.835
54	Insecticide	Phenyl isocyanate	1030	53.96	178.0509	3.935
55	Fungicide	Myclobutanil	1009	50.56	287.1061	7.333
56	Pesticide	Ancymidol	1003	75.70	255.1122	5.734
Maize 5						

Nr	Class	Name	Abundance	Score	m/z	RT (min)
1	Fungicide	Flusilazol	321282	73.32	314.0956	3.846
2	Fungicide	Anilazine	254725	53.04	272.9486	0.449
3	Insecticide	Cevadin	74893	61.50	636.3344	3.247
4	Herbicide	Dinoseb-acetate	39220	84.08	341.0966	0.699
5	Herbicide	Medinoterbacetate	39220	84.08	341.0966	0.699
6	Fungicide	Etridiazole	33952	52.11	306.9279	0.449
7	Insecticide	Isolan	10737	83.48	210.1267	3.097
8	Pesticide	2,4-Dinitrophenol	8948	79.38	183.0040	6.994
9	Fungicide	Furmecyclox	8502	59.78	250.1452	6.944
10	Insecticide	Tebufenozide	7744	82.38	397.2131	7.444
11	Insecticide	Demeton-O	7233	78.41	257.0433	3.097
12	Herbicide	Diethofencarb	6426	54.61	266.1404	7.294
13	Insecticide	Disulfoton	6404	57.70	273.0232	1.848
14	Acaricide	Aminocarb	6356	67.94	267.1373	7.844
15	Herbicide	Diethofencarb	5324	56.90	266.1406	3.646
16	Insecticide	Carbosulfan	4906	56.24	379.2033	7.844
17	Fungicide	Chlozolinate	4800	56.71	390.0119	7.144
18	Insecticide	Bomyl	3998	71.47	327.0508	4.845
19	Fungicide	Folpet	3994	53.97	341.8981	0.499
20	Pesticide	4,4'-Bipyridyl	3163	77.84	215.0826	3.097
21	Acaricide	Aminocarb	3004	75.40	267.1361	7.094
22	Pesticide	Ancymidol	2986	56.61	255.1128	6.894
23	Herbicide	Cyprazine	2717	54.26	286.1088	7.394
24	Fungicide	Isazofos	2121	65.85	372.0532	3.846
25	Herbicide	Aziprotryne	1792	51.34	284.0934	6.894
26	Insecticide	Fenazaquin	1678	70.37	365.1887	7.344
27	Insecticide	Dimetilan	1656	67.17	299.1378	3.197
28	Plant growth regulator	Thidiazuron	1535	52.15	219.0337	3.097
29	Plant growth regulator	Dikegulac	1438	65.64	319.1065	3.147
30	Herbicide	Deiquat	1412	81.55	183.0938	3.147
31	Insecticide	Ciodrin (Crotoxyphos)	1282	51.81	359.0932	3.347
32	Acaricide	Phenkapton	1251	58.81	420.9342	7.144
33	Fungicide	Triamiphos	1208	67.62	293.1302	6.844
34	Fungicide	Etaconazole	1202	70.78	372.0528	3.846
35	Herbicide	Diflufenzopyr	1188	59.80	333.0811	3.347
36	Herbicide	Fosamin-ammonium	1178	50.62	212.0315	3.097
37	Herbicide	Bentazone	1169	62.29	239.0519	7.444
38	Insecticide	Tebupirimfos	1168	65.03	317.1074	3.047
39	Fungicide	Cymoxanil (Curzate)	1165	71.08	257.0903	3.197
40	Herbicide	Orbencarb	1135	51.98	316.0791	3.147
41	Fungicide	Pencycuron	1105	73.24	327.1253	3.347
42	Insecticide	Trimethacarb	1009	57.11	252.1249	7.344
Maize 6						

Nr	Class	Name	Abundance	Score	m/z	RT (min)
1	Insecticide	Tebufenozide	64534	80.66	351.2088	6.944
2	Insecticide	Cevadin	36347	66.52	636.3348	3.246
3	Insecticide	Flufenoxuron	25089	58.26	533.0353	5.545
4	Herbicide	Dinoseb-acetate	18284	82.43	341.0966	0.848
5	Herbicide	Medinoterbacetate	14812	82.57	341.0967	0.848
6	Pesticide	2,4-Dinitrophenol	14400	78.08	183.0040	6.994
7	Fungicide	Etridiazole	12416	50.33	306.9277	0.448
8	Herbicide	Chloroxuron	9583	54.56	335.0803	6.944
9	Insecticide	Tebufenozide	6483	80.10	397.2143	7.344
10	Fungicide	Hexaconazole	6411	58.83	312.0660	6.744
11	Insecticide	Disulfoton	6279	51.33	273.0235	1.847
12	Acaricide	Aminocarb	5529	78.09	267.1363	7.993
13	Insecticide	Fenazaquin	5291	64.98	351.1740	7.294
14	Herbicide	Diethofencarb	5003	58.41	266.1405	4.096
15	Herbicide	MCPA-thioethyl	4953	58.82	243.0234	2.347
16	Fungicide	Furmecyclox	4881	51.00	250.1444	6.994
17	Insecticide	Carbosulfan	4148	72.09	379.2051	7.843
18	Insecticide	Isofenphos	4143	62.47	344.1054	4.345
19	Insecticide	Carbaryl	4061	52.09	260.0942	6.694
20	Fungicide	Triamiphos	3968	50.50	293.1294	6.944
21	Fungicide	2,3-Dichlorophenol	3298	51.49	206.9635	8.193
22	Herbicide	Cyanazine	3219	50.75	239.0809	3.146
23	Pesticide	Methiocarb-sulfoxide	3127	64.86	286.0724	3.496
24	Fungicide	Cymoxanil (Curzate)	2764	67.27	257.0902	3.096
25	Acaricide	Aminocarb	2759	70.30	267.1370	3.546
26	Pesticide	4,4'-Bipyridyl	2607	83.86	215.0828	3.096
27	Fungicide	Trifloxystrobin	2589	67.63	407.1244	3.246
28	Herbicide	Deiquat	2474	85.57	243.1134	3.196
29	Acaricide	Aminocarb	2460	78.22	267.1364	7.294
30	Fungicide	Pencycuron	2381	68.19	327.1289	3.296
31	Fungicide	Chlozolinate	1823	54.02	390.0121	7.144
32	Fungicide	2,3-Dichlorophenol	1696	51.69	206.9646	2.297
33	Fungicide	Benomyl	1680	59.03	349.1521	3.646
34	Acaricide	Dinobuton	1644	71.07	325.1025	5.095
35	Herbicide	Pyridate	1543	59.41	377.1095	3.196
36	Insecticide	Dimetilan	1531	78.95	299.1376	3.546
37	Insecticide	Fluenethyl	1292	50.34	317.1169	3.146
38	Fungicide	Fuberidazole	1255	66.58	183.0551	3.046
39	Plant growth regulator	Thidiazuron	1249	69.60	219.0338	3.096
40	Fungicide	Ziram	1221	52.43	362.9331	0.948
41	Insecticide	Demeton-O	1146	58.55	257.0426	3.896
42	Herbicide	Oxasulfuron	1121	56.59	465.1081	3.146
43	Herbicide	Dalapon	1107	63.46	200.9738	0.648
44	Fungicide	Quassin	1106	55.05	449.2172	3.196

Nr	Class	Name	Abundance	Score	m/z	RT (min)
45	Fungicide	Pyrazophos	1104	72.15	372.0792	3.096
46	Herbicide	Propaquizafop	1104	52.04	442.1156	7.993
47	Insecticide	Fenchlorphos	1026	61.79	378.9111	3.546
48	Herbicide	Bensulfuron-methyl	1025	56.20	409.0789	6.794
49	Pesticide	Ancymidol	1022	81.50	255.1119	6.894
Maize 7						
1	Insecticide	Cevadin	59919	64.03	636.3349	3.246
2	Herbicide	Dinoseb-acetate	46903	85.59	341.0968	0.698
3	Herbicide	Chloroxuron	36319	66.10	335.0810	6.944
4	herbicide	Bromofenoxim	9718	55.48	519.8805	0.448
5	Insecticide	Tebufenozide	8264	80.70	351.2074	6.894
6	Insecticide	Isolan	7176	72.19	210.1267	3.096
7	Herbicide	Medinoterbacetate	7060	87.28	341.0977	2.946
8	Insecticide	Disulfoton	6692	54.50	273.0233	1.897
9	Insecticide	DNOC (2,4-Dinitro-o-kresol)	6649	56.26	257.0427	3.096
10	Pesticide	2,4-Dinitrophenol	6192	77.07	183.0041	7.843
11	Fungicide	Furmecyclox	5964	57.44	250.1451	7.044
12	Herbicide	Diethofencarb	5574	52.94	266.1409	7.094
13	Pesticide	4,4'-Bipyridyl	5323	84.58	215.0833	3.146
14	Insecticide	Demeton-O	4735	55.81	257.0441	3.096
15	Acaricide	Aminocarb	4699	79.62	267.1361	7.843
16	Insecticide	Isofenphos	4414	64.15	344.1057	4.295
17	Acaricide	Aminocarb	4115	71.07	267.1364	7.094
18	Insecticide	Carbosulfan	4097	53.04	379.2044	6.894
19	Herbicide	Falone	3369	57.89	706.9318	7.244
20	Herbicide	Diethofencarb	3260	76.33	266.1406	3.596
21	Herbicide	Endothal	3237	59.06	245.0646	3.146
22	Fungicide	Folpet	2506	51.52	341.8964	0.498
23	Insecticide	Flufenoxuron	2488	60.41	533.0351	5.145
24	Insecticide	Fenazaquin	2420	64.73	351.1744	6.994
25	Fungicide	Flusilazol	2256	55.42	314.0950	3.846
26	Acaricide	Aminocarb	2190	69.95	267.1363	3.596
27	Herbicide	Chloridazone	2158	52.38	220.0263	3.396
28	Plant growth regulator	Dikegulac	1976	68.07	333.1217	3.396
29	Herbicide	Fluometuron	1838	56.09	231.0770	3.046
30	Herbicide	Aziprotryne	1760	52.83	284.0935	6.894
31	Herbicide	Carbetamide	1671	54.91	295.1319	3.496
32	Herbicide	Florasulam	1597	67.73	404.0266	7.194
33	Herbicide	Dinoseb	1595	82.54	299.0888	3.346
34	Insecticide	Diafenthiuron	1590	67.78	383.2165	6.944
35	Herbicide	Triflusulfuron-methyl	1554	59.77	537.1007	6.094
36	Fungicide	Cymoxanil (Curzate)	1531	72.13	257.0899	3.096
37	Insecticide	Prothoate	1461	72.10	330.0591	4.995

Nr	Class	Name	Abundance	Score	m/z	RT (min)
38	Insecticide	Dimetilan	1395	89.35	299.1376	3.196
39	Pesticide	Ancymidol	1341	74.36	255.1125	3.146
40	Fungicide	Oxadixyl	1323	57.43	337.1433	3.096
41	Fungicide	Iprobenfos	1282	57.66	347.1094	3.096
42	Pesticide	Cadusafos	1275	53.72	315.0849	3.146
43	Herbicide	Difenoxuron	1206	59.65	285.1215	3.046
44	Pesticide	Indoxacarb	1020	58.89	572.0695	7.893
45	Fungicide	Famoxadone	1000	68.17	373.1186	4.195
Maize 8						
1	Herbicide	Hexazinone	160495	91.92	311.1709	6.942
2	Insecticide	Cevadin	62655	62.23	636.3348	3.244
3	Herbicide	Medinoterbacetate	28103	83.99	341.0967	0.896
4	Herbicide	Dinoseb-acetate	23337	84.17	341.0967	0.896
5	Herbicide	Fluroxypyr	13560	58.14	312.9794	3.544
6	Herbicide	Ethofumesate	8028	52.54	345.0988	6.992
7	Herbicide	Fosamin-ammonium	7976	55.22	212.0349	3.194
8	Insecticide	Demeton-O	7593	53.78	257.0454	3.094
9	Fungicide	Triadimenol	5937	68.73	294.1024	6.093
10	Insecticide	Tebufenozide	5902	95.68	397.2133	7.392
11	Insecticide	Thanite	5628	67.52	298.1105	6.242
12	Insecticide	Tebufenozide	5602	84.10	351.2079	6.992
13	Pesticide	4,4'-Bipyridyl	5153	84.00	215.0819	3.094
14	Pesticide	2,4-Dinitrophenol	5013	78.33	183.0037	7.841
15	Acaricide	Aminocarb	4814	75.54	267.1360	7.841
16	Herbicide	Diethofencarb	4224	54.26	266.1402	4.093
17	Herbicide	Florasulam	3882	68.59	404.0267	7.192
18	Fungicide	Furmecyclox	3360	51.60	250.1444	7.042
19	Insecticide	Fenazaquin	3052	78.74	351.1714	6.992
20	Fungicide	Quinoxyfen	2864	52.51	366.0094	6.942
21	Fungicide	Fluazinam	2785	59.38	508.9535	7.092
22	Fungicide	Pyrifenox	2425	55.61	293.0241	4.243
23	Fungicide	Cymoxanil (Curzate)	2285	73.58	257.0884	3.144
24	Insecticide	Diafenthiuron	2207	61.10	443.2399	3.094
25	Herbicide	Carbetamide	2201	64.28	295.1304	3.544
26	Fungicide	Trifloxystrobin	2181	54.69	467.1399	3.094
27	Herbicide	Fluroxypyr-meptyl	2146	60.09	365.0768	3.094
28	Acaricide	Aminocarb	2107	62.03	267.1377	7.042
29	Insecticide	Flufenoxuron	2087	58.95	533.0345	5.593
30	Pesticide	Ancymidol	2008	77.36	255.1129	3.144
31	Insecticide	Bendiocarb	1963	54.08	282.0991	3.444
32	Insecticide	5-Hydroxyimidacloprid	1846	80.98	270.0378	3.144
33	Fungicide	Myclobutanil	1834	51.51	347.1249	7.092
34	Herbicide	Cyprazine	1793	59.49	286.1086	7.392
35	Insecticide	Monocrotophos	1753	67.14	268.0594	5.393

Nr	Class	Name	Abundance	Score	m/z	RT (min)
36	Insecticide	Etrimfos	1661	54.86	351.0756	6.792
37	Herbicide	Defenurone	1557	81.88	195.0772	6.942
38	Herbicide	Isoxaben	1546	73.13	391.1878	6.892
39	Fungicide	Benomyl	1522	61.65	349.1509	3.294
40	Herbicide	Naptalam	1482	68.05	290.0834	4.343
41	Fungicide	Dimoxystrobin	1431	53.34	371.1643	3.994
42	Fungicide	Triamiphos	1395	71.38	353.1516	3.144
43	Herbicide	Atrazine	1380	66.32	260.0929	6.692
44	Pesticide	Isocarbophos	1370	56.85	288.0488	3.144
45	Pesticide	Cadusafos	1287	54.15	329.1027	3.294
46	acaricide	Fluazuron	1177	59.49	549.9999	0.596
47	Insecticide	Dimetilan	1169	82.50	285.1199	3.144
48	Plant growth regulator	Dikegulac	1164	56.03	333.1200	3.194
49	Herbicide	Flamprop-isopropyl	1162	55.56	422.1170	4.543
50	Insecticide	Pirimicarb	1158	70.48	283.1408	6.043
51	Herbicide	Naphthoxyacetic acid	1113	64.88	201.0582	5.843
52	Herbicide	Simazine	1103	57.22	260.0930	6.842
53	Fungicide	Benomyl	1096	60.23	349.1521	6.892
54	Herbicide	Metamitron	1043	69.65	247.0853	3.994
Maize 9						
1	Herbicide	Methabenzthiazuron	917876	67.59	244.0497	13.535
2	Herbicide	Tepraloxydim	145944	61.28	359.1751	5.291
3	Pesticide	Aldicarb-sulfone (Aldoxycarb)	114746	80.91	240.0993	13.535
4	Herbicide	Trifluralin	93609	91.01	336.1159	13.036
5	Insecticide	Quintiofos	91706	71.34	330.0725	14.235
6	Insecticide	Benfuracarb	57735	79.38	428.2214	5.791
7	Herbicide	Sethoxydim	53206	78.77	350.1784	0.943
8	Insecticide	Fenpropathrin	53206	70.03	350.1784	0.943
9	Pesticide	Pyridaben	48054	53.57	365.1473	0.893
10	Fungicide	8-Hydroxychinolin	42946	78.45	168.0433	13.585
11	Acaricide	Fenpyroximate	34478	61.06	439.2313	1.393
12	Fungicide	Pyrazophos	31298	70.40	391.1199	15.934
13	Insecticide	Pyrethrin I	28112	52.35	351.1908	5.291
14	Fungicide	Dichlofluanid	19147	94.04	349.9970	13.535
15	Fungicide	Isazofos	18377	67.36	331.0756	14.235
16	Insecticide	Barthrin	12867	57.79	337.1197	13.036
17	Herbicide	Daminozide	11527	80.37	161.0916	15.984
18	Herbicide	Fluridone	7085	72.54	352.0898	13.036
19	Insecticide	Mipafox	5165	90.87	205.0864	1.143
20	Insecticide	Cevadin	5088	90.59	614.3305	5.441
21	Insecticide	Bendiocarb	4977	54.58	241.1166	15.934
22	Insecticide	Dimetilan	3991	84.40	263.1119	0.693
23	Fungicide	Chlozolinate	3918	70.18	353.9913	13.535

Nr	Class	Name	Abundance	Score	m/z	RT (min)
24	Insecticide	Dinocap	3656	71.76	365.1690	0.843
25	Herbicide	Piperophos	3534	67.00	371.1618	5.141
26	Insecticide	Diafenthiuron	3500	57.69	385.2308	5.591
27	Insecticide	Jasmolin I	3125	84.49	353.2088	1.793
28	Herbicide	Buturon	2980	58.73	254.1058	15.034
29	Herbicide	Dicryl	2882	51.81	247.0400	13.985
30	Herbicide	Metribuzin	2764	55.87	232.1241	5.441
31	Herbicide	Flazasulfuron	2586	68.99	408.0603	15.934
32	Insecticide	Jasmolin I	2380	97.35	353.2090	2.392
33	Herbicide	Methoxyphenone	2130	78.66	241.1227	1.793
34	Fungicide	Picoxystrobin	2004	71.50	385.1397	6.890
35	Herbicide	Atraton	1996	74.90	212.1514	2.692
36	Herbicide	Lambast	1979	55.45	324.1433	13.985
37	Herbicide	Diethofencarb	1922	73.09	285.1809	15.834
38	Insecticide	Jasmolin I	1870	78.36	353.2087	5.291
39	Herbicide	Dinoseb-acetate	1849	56.47	283.0904	13.835
40	Insecticide	Azinphos-ethyl	1838	75.46	346.0458	13.585
41	Insecticide	Lethane 384	1803	92.63	221.1327	2.342
42	Herbicide	Propanil	1755	50.31	235.0396	13.835
43	Herbicide	Carbetamide	1742	67.29	259.1041	0.843
44	Pesticide	Maleic hydrazide (MH)	1683	57.18	130.0612	0.693
45	Herbicide	Naphthoxyacetic acid	1648	52.32	203.0714	0.843
46	Insecticide	Jasmolin I	1641	75.79	353.2088	3.142
47	Insecticide	Phoxim	1634	69.76	299.0605	6.990
48	Fungicide	Bupirimate	1626	62.09	317.1650	15.784
49	Insecticide	Jasmolin I	1590	75.57	353.2083	1.393
50	Insecticide	MNFA	1532	81.45	235.1243	13.185
51	Herbicide	Imazamox	1517	60.07	323.1736	13.135
52	Herbicide	Naptalam	1507	75.20	309.1239	13.086
53	Insecticide	Fenazaquin	1498	61.43	307.1796	13.086
54	Herbicide	Profluralin	1491	82.45	365.1403	3.142
55	Fungicide	Propamocarb	1471	77.55	189.1583	15.934
56	Insecticide	Thanite	1443	70.05	271.1477	16.134
57	Fungicide	Benomyl	1406	79.86	291.1465	13.235
58	Herbicide	Fluorodifen	1403	53.47	346.0675	13.185
59	Fungicide	Cymoxanil (Curzate)	1369	57.60	199.0826	2.642
60	Insecticide	Phenothrin	1347	54.83	351.1990	14.085
61	Fungicide	Bupirimate	1329	73.70	317.1633	13.785
62	Herbicide	Methoxyphenone	1302	72.15	263.1045	5.090
63	Herbicide	Fenaclon	1290	56.93	212.0839	8.838
64	Herbicide	Cinidon-ethyl	1262	61.80	411.0892	0.793
65	Herbicide	Quinclorac	1238	50.22	241.9755	0.494
66	Herbicide	Imazethapyr	1228	57.24	307.1788	13.185
67	Insecticide	Terbufos	1178	50.74	306.0799	9.888

Nr	Class	Name	Abundance	Score	m/z	RT (min)
68	Plant growth regulator	Azoluron	1164	66.41	253.1060	13.235
69	Herbicide	Imazaquin	1148	75.70	329.1598	14.385
70	Insecticide	Aldicarb	1115	64.80	213.0675	5.541
71	Herbicide	Pretilachlor	1107	62.75	329.1982	0.893
72	Insecticide	Tebufenozide	1087	60.60	375.2025	13.135
73	Plant growth regulator	Trinexapac-ethyl	1079	81.54	253.1061	13.535
74	Insecticide	Ethoprofos	1057	61.61	260.0910	15.034
75	Fungicide	Fludioxonil	1034	85.37	249.0469	13.535
76	Fungicide	Oxycarboxin	1021	78.21	290.0443	3.841
77	Insecticide	Flucythrinate	1000	66.41	469.1932	1.143
Maize 10						
1	Insecticide	Isolan	87870	82.77	210.1269	3.094
2	Insecticide	Cevadin	66308	60.90	636.3349	3.244
3	Herbicide	Dinoseb-acetate	31275	84.68	341.0967	0.746
4	Herbicide	Medinoterbacetate	31275	84.68	341.0967	0.746
5	Herbicide	Bromofenoxim	18026	59.04	519.8818	0.446
6	Insecticide	Tebufenozide	14237	76.55	351.2066	6.942
7	Insecticide	Demeton-O	14145	75.13	257.0434	3.044
8	Fungicide	Etridiazole	9572	55.94	306.9286	0.446
9	Insecticide	Tebufenozide	8664	78.26	397.2133	7.441
10	Pesticide	2,4-Dinitrophenol	7630	74.26	183.0038	6.992
11	Herbicide	Falone	6179	65.52	708.9281	7.241
12	Insecticide	Dimetilan	6039	85.12	285.1207	3.544
13	Fungicide	Furmecyclox	5547	56.30	250.1455	7.042
14	Insecticide	Carbosulfan	5231	59.32	379.2040	7.841
15	Herbicide	Diethofencarb	5081	57.66	266.1404	3.943
16	Insecticide	Bomyl	4924	59.65	327.0512	4.893
17	Herbicide	Chloroxuron	4896	59.34	335.0805	6.942
18	Insecticide	Flufenoxuron	3748	56.78	533.0358	5.643
19	Herbicide	Naptalam	3236	85.41	290.0821	4.343
20	Herbicide	Diflufenican	3090	56.47	393.0717	3.094
21	Fungicide	Triadimenol	2984	53.94	294.1020	6.142
22	Herbicide	Picolinafen	2619	53.28	375.0742	7.241
23	Insecticide	Thiofanox	2580	58.54	217.0985	3.194
24	Herbicide	MCPA-thioethyl	2405	63.02	243.0239	1.695
25	Insecticide	DNOC (2,4-Dinitro-o-kresol)	2405	60.66	243.0239	1.695
26	Plant growth regulator	Trinexapac-ethyl	2333	66.12	297.1008	4.393
27	Fungicide	Iprobenfos	2315	54.74	347.1110	3.044
28	Insecticide	Clenpirin	2315	53.41	343.1000	3.344
29	Herbicide	Ethofumesate	2275	53.65	285.0792	3.144
30	Pesticide	4,4'-Bipyridyl	2240	69.56	215.0823	3.144
31	Fungicide	Quinoxyfen	2140	51.98	366.0093	6.942

Nr	Class	Name	Abundance	Score	m/z	RT (min)
32	Fungicide	Cymoxanil (Curzate)	2012	76.62	257.0902	3.094
33	Herbicide	Cyprazine	1956	57.80	286.1097	7.341
34	Fungicide	Pencycuron	1946	60.42	327.1295	3.294
35	Insecticide	Monocrotophos	1889	76.09	268.0591	5.443
36	Insecticide	Ethoprofos	1858	51.64	301.0681	3.094
37	Herbicide	Fosamin-ammonium	1683	51.99	212.0331	3.294
38	Herbicide	Bentazone	1562	59.69	239.0488	3.094
39	Herbicide	Prodiamine	1553	57.30	349.1139	3.094
40	Fungicide	Benomyl	1546	55.36	335.1359	3.144
41	Fungicide	Triamiphos	1463	63.17	353.1496	3.244
42	Herbicide	Orbencarb	1440	53.20	316.0767	3.144
43	Insecticide	Carbaryl	1407	53.04	260.0925	6.642
44	Plant growth regulator	Dikegulac	1397	59.29	333.1182	3.094
45	Herbicide	Deiquat	1393	80.03	229.0984	3.194
46	Insecticide	Fenazaquin	1361	70.68	305.1643	6.492
47	Insecticide	5-Hydroxyimidacloprid	1351	54.58	330.0591	3.094
48	Fungicide	Flusilazol	1271	50.45	314.0959	3.793
49	Herbicide	Atrazine	1231	57.43	260.0925	6.842
50	Herbicide	Aziprotryne	1164	82.36	270.0767	5.293
51	Insecticide	Dibutyldimethylbenzylphenol	1155	54.54	369.2450	7.042
52	Herbicide	Simazine	1094	57.43	260.0925	6.842
53	Fungicide	Fenoxanil	1059	54.20	327.0695	3.993
54	Insecticide	Pirimicarb	1030	74.31	283.1412	3.194
Maize 11						
1	Fungicide	Flusilazol	691057	69.36	314.0957	3.837
2	Herbicide	Hexazinone	146815	81.24	311.1703	6.945
3	Herbicide	Orbencarb	132026	54.47	256.0550	3.137
4	Herbicide	Trifluralin	129831	89.00	336.1161	13.038
5	Herbicide	Tepraloxydim	109581	61.25	359.1752	5.293
6	Insecticide	Cevadin	64639	84.47	592.3488	4.343
7	Insecticide	Cevadin	61343	59.93	636.3339	3.247
8	Herbicide	Sethoxydim	45425	58.88	350.1787	0.946
9	Insecticide	Benfuracarb	34196	78.85	428.2213	5.793
10	Herbicide	Medinoterbacetate	30286	80.70	341.0963	0.649
11	Insecticide	Prothoate	29601	55.60	330.0585	5.036
12	Acaricide	Fenpyroximate	28960	50.06	439.2314	1.445
13	Herbicide	Dinoseb-acetate	28705	87.75	341.0969	2.937
14	Herbicide	Medinoterbacetate	28705	87.75	341.0969	2.937
15	Herbicide	Dinoseb-acetate	27288	80.41	341.0963	0.649
16	Fungicide	Flusilazol	26777	77.13	314.0945	3.847
17	Herbicide	Diflufenican	26437	82.01	439.0704	0.599
18	Insecticide	Pyrethrin I	25446	55.30	351.1912	5.293
19	Herbicide	Bromofenoxim	21163	57.96	519.8816	0.439

Nr	Class	Name	Abundance	Score	m/z	RT (min)
20	Insecticide	5-Hydroxyimidacloprid	20725	53.30	330.0585	5.036
21	Insecticide	DNOC (2,4-Dinitro-o-kresol)	16493	60.88	257.0424	3.087
22	Herbicide	Fluridone	15955	71.56	352.0896	13.038
23	Herbicide	Foramsulfuron	14496	81.21	511.1248	6.735
24	Insecticide	Isolan	12903	87.03	210.1266	3.097
25	Herbicide	Chloroxuron	9758	58.80	335.0807	5.936
26	Insecticide	Etrimfos	9442	50.67	291.0544	6.995
27	Fungicide	Methfuroxam	8267	80.62	252.0990	8.041
28	Herbicide	Propazine	8267	61.88	252.0990	8.041
29	Herbicide	Flamprop-isopropyl	7908	66.41	364.1103	6.992
30	Insecticide	Isolan	7892	69.29	210.1270	3.087
31	Insecticide	Tebufenozide	7874	80.04	351.2077	6.935
32	Acaricide	Aminocarb	7006	87.72	267.1369	7.934
33	Insecticide	Lethane 60	6872	50.66	308.1634	5.693
34	Acaricide	Aminocarb	6852	92.68	267.1364	7.944
35	Insecticide	Tebufenozide	5957	82.05	397.2131	7.445
36	Insecticide	Dinocap	5865	97.14	365.1703	0.846
37	Herbicide	Diethofencarb	5573	58.04	266.1403	7.345
38	Insecticide	Diafenthiuron	5479	51.00	407.2113	5.593
39	Herbicide	Imazapyr	5277	86.66	284.1012	0.746
40	Insecticide	Fenazaquin	5268	81.26	351.1725	7.345
41	Pesticide	2,4-Dinitrophenol	5199	80.40	183.0042	6.985
42	Insecticide	Ethoprofos	5002	58.13	241.0479	3.487
43	Insecticide	Flufenoxuron	4933	53.91	533.0359	5.536
44	Insecticide	Bomyl	4347	53.52	327.0505	4.846
45	Insecticide	Tebufenozide	4217	86.83	397.2137	7.435
46	Herbicide	Dicryl	4154	58.16	247.0394	13.988
47	Insecticide	Fonofos	4154	54.40	247.0394	13.988
48	Insecticide	Phenyl isocyanate	4000	75.20	178.0509	3.936
49	Pesticide	2,4-Dinitrophenol	3970	58.05	183.0036	7.045
50	Herbicide	Diethofencarb	3963	51.49	266.1404	4.236
51	Fungicide	Picoxystrobin	3760	75.92	385.1386	6.892
52	Herbicide	MCPA-thioethyl	3674	61.90	243.0238	1.688
53	Herbicide	Simetryn	3671	60.45	212.0986	6.545
54	Herbicide	Chlorbromuron	3592	53.38	294.9651	2.295
55	Herbicide	Cinidon-ethyl	3355	57.80	411.0894	0.446
56	Herbicide	Cycluron	3352	70.42	243.1695	4.936
57	Herbicide	Cyprazine	3149	57.94	286.1082	7.345
58	Plant growth regulator	Dikegulac	3073	67.73	333.1206	3.187
59	Insecticide	Lethane 384	3054	80.70	221.1327	1.445
60	Acaricide	Aminocarb	2897	76.12	267.1363	7.045
61	Herbicide	Neburon	2840	58.62	273.0565	5.536
62	Pesticide	Pyridaben	2718	56.92	423.1454	3.237

Nr	Class	Name	Abundance	Score	m/z	RT (min)
63	Insecticide	Mipafox	2715	74.36	205.0880	1.895
64	Fungicide	Benomyl	2692	62.48	349.1499	3.437
65	Pesticide	4,4'-Bipyridyl	2675	75.42	215.0811	3.097
66	Herbicide	Desmedipham	2668	71.33	359.1244	7.095
67	Herbicide	Buturon	2597	52.56	254.1060	15.037
68	Herbicide	Aclonifen	2480	51.12	323.0454	4.686
69	Pesticide	4,4'-Bipyridyl	2478	61.06	215.0829	3.087
70	Fungicide	Binapacryl	2452	60.63	367.1159	3.287
71	Herbicide	Buminafos	2355	69.22	370.2506	5.643
72	Insecticide	Tebufenozide	2334	78.21	370.2503	5.593
73	Insecticide	Jasmolin I	2324	76.50	353.2099	5.443
74	Insecticide	Deguelin	2317	87.94	412.1767	1.146
75	Insecticide	Monocrotophos	2303	67.12	268.0594	5.386
76	Fungicide	Famoxadone	2283	65.33	373.1194	4.186
77	Pesticide	Maleic hydrazide (MH)	2177	61.54	130.0611	0.696
78	Insecticide	Dimetilan	2167	66.22	285.1206	3.237
79	Insecticide	Jasmolin I	2165	81.70	353.2076	2.395
80	Herbicide	Desmedipham	2136	65.26	359.1225	3.537
81	Herbicide	Medinoterbacetate	2090	81.99	319.0889	13.788
82	Fungicide	Etridiazole	2083	60.92	306.9323	4.086
83	Insecticide	Phorate	2065	73.90	278.0459	7.992
84	Fungicide	Bupirimate	2037	68.81	317.1658	6.892
85	Fungicide	Iprobenfos	2032	58.76	287.0886	4.986
86	Herbicide	Deiquat	2019	79.34	183.0940	3.387
87	Insecticide	Heptenophos	2011	54.22	309.0313	3.737
88	Herbicide	Imazethapyr	1911	63.57	307.1787	13.538
89	Pesticide	Phorate-sulphoxide	1855	75.53	294.0402	13.038
90	Herbicide	Desmedipham	1848	60.49	301.1196	14.088
91	Herbicide	Orbencarb	1845	50.35	258.0707	5.893
92	Insecticide	Allethrin II	1834	69.71	391.1736	6.945
93	Fungicide	Triamiphos	1830	66.46	293.1287	6.885
94	Insecticide	Carbosulfan	1811	70.55	379.2063	7.884
95	Herbicide	Dinoseb-acetate	1800	93.36	283.0922	13.588
96	Pesticide	Aldicarb-sulfone (Aldoxycarb)	1799	64.66	245.0547	0.946
97	Fungicide	Fenamidone	1783	83.48	334.1009	4.993
98	Herbicide	Fosamin-ammonium	1768	63.07	212.0345	4.786
99	Insecticide	Acrinathrin	1764	69.14	540.1224	5.336
100	Insecticide	Hydramethylnon	1760	59.46	493.1804	3.437
101	Herbicide	Chloridazone	1747	50.13	280.0477	3.387
102	Insecticide	Thiofanox	1736	77.01	241.0983	4.144
103	Herbicide	Ethofumesate	1732	50.16	309.0803	9.840
104	Insecticide	Fenazaquin	1721	76.56	351.1728	7.335
105	Plant growth regulator	Trinexapac-ethyl	1647	56.67	251.0949	3.597

Nr	Class	Name	Abundance	Score	m/z	RT (min)
106	Fungicide	Quassin	1642	61.51	449.2195	3.437
107	Fungicide	Isazofos	1615	61.37	331.0735	7.142
108	Herbicide	Imazamox	1597	61.50	323.1726	13.388
109	Herbicide	Oryzalin	1571	67.13	391.0926	5.286
110	Herbicide	Atrazine-desethyl-desisopropyl	1552	59.52	204.0294	3.247
111	Fungicide	Benomyl	1548	62.96	335.1367	6.785
112	Insecticide	Dinocap	1539	66.03	423.1776	6.995
113	Fungicide	Diniconazole	1534	57.61	343.1107	11.390
114	Herbicide	Difenoxuron	1533	55.41	285.1210	3.397
115	Herbicide	Fluometuron	1487	66.72	231.0745	3.147
116	Herbicide	Cyanazine	1454	55.39	239.0804	3.147
117	Herbicide	Picolinafen	1435	58.71	375.0753	5.586
118	Insecticide	Jasmolin I	1423	84.32	353.2085	1.795
119	Herbicide	Aziprotryne	1409	60.00	270.0775	5.346
120	Herbicide	Methoxyphenone	1408	71.42	241.1226	1.845
121	Herbicide	Triflusulfuron-methyl	1402	72.14	537.1040	6.036
122	Insecticide	Tebufenozide	1378	67.61	370.2511	5.443
123	Insecticide	Bromophos-ethyl	1375	56.04	416.8686	0.446
124	Acaricide	Methiocarb-sulfoxide	1336	52.80	259.1134	9.191
125	Insecticide	Fluenethyl	1336	62.90	259.1134	9.191
126	Fungicide	Chloranilormethan	1317	57.89	380.8934	7.085
127	Fungicide	Triamiphos	1298	74.90	295.1419	5.843
128	Herbicide	Chloral methyl hemiacetal	1291	66.51	238.9465	6.895
129	Herbicide	Profluralin	1273	63.78	365.1402	2.395
130	Herbicide	Oxasulfuron	1271	62.34	407.1007	5.543
131	Pesticide	Aldicarb-sulfone (Aldoxycarb)	1235	51.50	221.0573	0.689
132	Insecticide	Methoxyfenozide	1219	68.85	386.2461	2.745
133	Fungicide	Drazoxolon	1207	57.00	282.0294	4.636
134	Insecticide	Bendiocarb	1198	70.41	241.1171	15.937
135	Fungicide	Etaconazole	1197	59.22	345.0880	13.088
136	Fungicide	Myclobutanil	1196	50.23	287.1079	7.395
137	Herbicide	Oxadiargyl	1154	51.93	385.0371	6.445
138	Herbicide	Flurtamone	1150	55.04	334.1011	4.993
139	Plant growth regulator	Dikegulac	1140	82.76	333.1212	8.044
140	Insecticide	Tinox	1129	64.27	275.0181	6.845
141	Insecticide	Demeton-O-methyl	1129	64.27	275.0181	6.845
142	Herbicide	Sulfometuron-methyl	1122	53.29	365.0939	13.888
143	Acaricide	Aminocarb	1112	67.20	267.1360	7.035
144	Fungicide	Dimoxystrobin	1105	69.05	349.1525	13.588
145	Herbicide	Erbon	1105	56.04	424.9089	7.145
146	Insecticide	Tetrachlorvinphos	1105	52.92	424.9089	7.145
147	Fungicide	Hexaconazole	1092	50.01	331.1076	6.992
148	Herbicide	Deiquat	1092	76.08	183.0936	3.497

Nr	Class	Name	Abundance	Score	m/z	RT (min)
149	Fungicide	Benomyl	1084	77.34	291.1443	13.938
150	Fungicide	Binapacryl	1060	63.03	345.1070	6.443
151	Herbicide	Simetryn	1053	51.96	214.1101	7.592
152	Fungicide	Thiophanate-methyl	1048	52.05	360.0791	5.593
153	Herbicide	Ethalfluralin	1047	71.22	334.1007	4.993
154	Insecticide	Flucythrinate	1044	63.45	469.1925	1.196
155	Insecticide	Novaluron	1041	58.92	551.0266	0.789
156	Fungicide	Bupirimate	1030	55.82	361.1539	3.647
157	Insecticide	Demeton-O-methyl	1027	53.62	248.0536	13.288
158	Herbicide	Metribuzin	1023	71.94	273.1027	4.796
159	Plant growth regulator	Azoluron	1019	77.25	275.1140	6.885
160	Herbicide	Diuron	1009	58.61	250.0506	7.042
Maize 12						
1	Insecticide	Cevadin	131686	68.63	636.3357	3.232
2	Fungicide	Anilazine	84744	54.51	272.9485	0.434
3	Insecticide	Carbaryl	71385	51.31	260.0942	6.530
4	Herbicide	Medinoterbacetate	34120	86.46	341.0970	0.684
5	Herbicide	Dinoseb-acetate	34120	86.46	341.0970	0.734
6	Fungicide	Etridiazole	18653	51.87	306.9279	0.484
7	Acaricide	Aminocarb	16684	86.86	267.1369	7.929
8	Herbicide	3,4-Dichloraniline	12891	58.34	159.9723	0.434
9	Pesticide	4,4'-Bipyridyl	12855	86.91	215.0829	3.082
10	Herbicide	Dinoseb	12541	68.35	285.0715	3.332
11	Insecticide	Tebufenozide	12262	80.89	351.2068	6.830
12	Insecticide	DNOC (2,4-Dinitro-o-kresol)	11698	74.23	257.0416	3.082
13	Herbicide	Bentazone	11638	60.71	239.0478	3.032
14	Pesticide	2,4-Dinitrophenol	9195	89.75	183.0043	6.880
15	Herbicide	Falone	7167	67.55	708.9307	7.180
16	Insecticide	Fluenethyl	6819	89.28	303.1027	6.580
17	Fungicide	Cymoxanil (Curzate)	6462	75.73	257.0889	3.082
18	Insecticide	Bromfenvinphos-methyl	6451	50.10	420.8825	0.434
19	Herbicide	Isoxaben	6181	53.41	331.1636	3.182
20	Fungicide	Fenarimol	5685	69.19	389.0463	6.480
21	Herbicide	Cycluron	5188	66.90	243.1697	4.931
22	Herbicide	Difenoxuron	5147	68.75	285.1228	3.082
23	Herbicide	Deiquat	4631	77.42	243.1131	3.132
24	Insecticide	Tebufenozide	4363	66.28	397.2128	7.330
25	Insecticide	Fenazaquin	4125	78.07	365.1876	7.879
26	Insecticide	Dimetilan	3879	73.37	285.1223	3.182
27	Insecticide	Ethoprofos	3727	57.61	301.0670	3.182
28	Insecticide	Carbosulfan	3708	63.28	379.2048	7.879
29	Herbicide	Mefenacet	3641	59.01	297.0697	3.832
30	Herbicide	Aziprotryne	3543	55.50	270.0775	5.231

Nr	Class	Name	Abundance	Score	m/z	RT (min)
31	Herbicide	Isomethiozin	3447	69.26	313.1347	6.580
32	Fungicide	Hexaconazole	3425	70.42	312.0673	6.780
33	Fungicide	Furmecyclox	2915	56.80	250.1457	6.930
34	Fungicide	Hexythiazox	2825	65.35	351.0945	3.132
35	Insecticide	Barthrin	2793	55.74	335.1066	3.132
36	Acaricide	Aminocarb	2734	76.02	267.1358	3.932
37	Insecticide	Tebupirimfos	2528	51.21	363.1165	3.682
38	Insecticide	Pymetrozine	2505	64.18	262.0921	6.530
39	Insecticide	Nitenpyram	2420	54.06	315.0844	3.132
40	Plant growth regulator	Dikegulac	2380	75.67	333.1200	7.929
41	Fungicide	Triamiphos	2242	67.76	293.1305	6.430
42	Acaricide	Methiocarb-sulfoxide	1973	62.23	300.0926	3.132
43	Insecticide	Monocrotophos	1877	70.67	282.0732	0.684
44	Fungicide	Pencycuron	1867	50.91	373.1342	3.182
45	Insecticide	MNFA	1864	56.72	262.0917	6.530
46	Fungicide	Benomyl	1650	56.18	349.1507	3.682
47	Herbicide	Terbacil	1642	61.50	261.0670	3.932
48	Herbicide	Bentranil	1628	50.13	282.0733	0.684
49	Insecticide	Dicrotophos Bildrin	1559	59.97	282.0736	0.684
50	Insecticide	Etrimfos	1559	53.37	291.0556	6.780
51	Fungicide	Bisphenol A	1549	68.93	287.1297	6.930
52	Plant growth regulator	Thidiazuron	1522	60.00	279.0539	6.880
53	Fungicide	Bupirimate	1503	63.13	375.1687	6.880
54	Pesticide	4,4'-Bipyridyl	1399	62.28	201.0684	1.434
55	Acaricide	Aminocarb	1304	80.51	267.1360	4.581
56	Herbicide	Metribuzin	1279	69.84	259.0865	4.232
57	Herbicide	Imazethapyr	1255	67.11	288.1351	1.484
58	Insecticide	Disulfoton	1231	51.01	273.0227	4.381
59	Insecticide	Dialifos	1211	65.97	391.9966	6.980
60	Herbicide	Monuron	1202	68.97	243.0525	0.684
61	Herbicide	Thiazafluron	1196	53.83	299.0455	4.631
62	Insecticide	Endosulfan sulfate	1182	59.11	466.8089	0.484
63	Herbicide	Trichloroacetic acid	1170	58.48	160.8964	0.634
64	Fungicide	Quassin	1129	69.08	449.2186	3.282
65	Herbicide	Pyrazosulfuron-ethyl	1126	56.87	459.0914	7.929
66	Fungicide	Phenylchlorophenol	1119	52.26	249.0328	6.830
67	Herbicide	MCPA-thioethyl	1077	60.53	243.0243	1.733
68	Insecticide	Pyrethrin I	1068	59.92	373.2046	3.082
69	Acaricide	Aminocarb	1057	65.00	267.1375	6.980
70	Fungicide	Cyazofamid	1047	58.01	323.0380	4.082
71	Herbicide	Dimefuron	1038	56.78	397.1258	3.132
Maize 13						
1	Fungicide	Anilazine	216925	56.42	272.9488	0.440

Nr	Class	Name	Abundance	Score	m/z	RT (min)
2	Fungicide	Etridiazole	89316	56.73	306.9287	0.490
3	Insecticide	Cevadin	65085	76.32	636.3363	3.238
4	Insecticide	Tebufenozide	47085	77.45	351.2089	6.835
5	Herbicide	Dinoseb-acetate	19063	88.89	341.0971	0.690
6	Herbicide	Medinoterbacetate	19063	88.89	341.0971	0.690
7	Insecticide	DNOC (2,4-Dinitro-o-kresol)	17274	83.76	257.0411	3.088
8	Pesticide	2,4-Dinitrophenol	16477	79.80	183.0041	6.935
9	Herbicide	Bromofenoxim	16249	57.72	519.8813	0.440
10	Acaricide	Aminocarb	11159	73.71	267.1365	7.935
11	Insecticide	Tebufenozide	8766	81.51	351.2081	7.935
12	Insecticide	Carbosulfan	8607	64.68	379.2045	6.935
13	Fungicide	Furmecyclox	8340	50.70	250.1463	6.935
14	Insecticide	Tebufenozide	7411	70.04	397.2121	7.385
15	Herbicide	Imazapyr	6992	94.29	306.1082	0.690
16	Herbicide	Dinoseb	6299	72.50	285.0709	3.487
17	Pesticide	Butocarboxim-sulphoxide	5661	55.94	251.0684	0.690
18	Fungicide	Folpet	5113	58.88	341.8980	0.490
19	Insecticide	Carbaryl	4254	52.20	260.0942	6.536
20	Herbicide	Hexazinone	4230	64.26	251.1500	6.985
21	Herbicide	Orbencarb	4158	65.53	256.0557	0.939
22	Herbicide	MCPA-thioethyl	3714	68.45	243.0246	2.338
23	Herbicide	Atrazine	3714	52.44	260.0941	6.536
24	Herbicide	Glyphosate	3485	60.82	228.0261	3.038
25	Insecticide	Azamethiphos	3455	56.93	322.9684	6.885
26	Herbicide	Cycluron	3252	71.45	243.1699	4.936
27	Herbicide	Diethofencarb	3091	52.89	266.1411	3.238
28	Insecticide	Thiacloprid	2870	61.35	251.0175	0.740
29	Acaricide	Aminocarb	2723	74.36	267.1364	3.138
30	Pesticide	Aldicarb-sulfone (Aldoxycarb)	2082	68.42	267.0668	0.690
31	Insecticide	5-Hydroxyimidacloprid	1966	56.14	330.0617	4.487
32	Herbicide	Cyanazine	1958	55.31	239.0815	3.188
33	Insecticide	Thiofanox	1840	50.75	217.0995	3.238
34	Pesticide	4,4'-Bipyridyl	1833	69.36	215.0831	3.088
35	Fungicide	Phenylchlorophenol	1805	60.26	249.0330	6.835
36	Acaricide	Aminocarb	1723	73.48	267.1365	7.035
37	Fungicide	Benomyl	1680	51.35	335.1380	3.537
38	Insecticide	Fluenethyl	1655	76.60	303.1024	6.636
39	Herbicide	Deiquat	1644	83.36	243.1130	3.337
40	Insecticide	Ethoprofos	1635	54.19	241.0478	3.487
41	Fungicide	Cymoxanil (Curzate)	1586	60.84	257.0911	3.138
42	Herbicide	Chlorphenprop-methyl	1525	57.78	231.0015	5.736
43	Herbicide	Prodiamine	1459	78.76	349.1140	6.985
44	Herbicide	Fosamin-ammonium	1413	81.91	212.0323	3.337

Nr	Class	Name	Abundance	Score	m/z	RT (min)
45	Herbicide	Cyprazine	1399	53.66	286.1097	7.335
46	Herbicide	Nitrofen	1293	55.79	341.9959	0.690
47	Insecticide	Dimetilan	1260	80.37	285.1220	3.337
48	Plant growth regulator	Dikegulac	1173	67.80	333.1214	7.935
49	Fungicide	Triamiphos	1165	75.59	293.1304	6.386
50	Plant growth regulator	Azoluron	1131	73.79	275.1151	6.885
51	Insecticide	Deguelin	1069	56.08	453.1536	3.088
52	Herbicide	Terbacil	1038	62.00	275.0814	6.835
53	Fungicide	Cyprodinil	1001	59.56	284.1395	7.935
Maize 14						
1	Insecticide	Tebufenozide	127271	77.00	351.2090	6.838
2	Insecticide	Cevadin	92738	66.30	636.3350	3.240
3	Herbicide	Dinoseb-acetate	55739	86.56	341.0969	0.692
4	Herbicide	Medinoterbacetate	55739	86.56	341.0969	0.692
5	Fungicide	Etridiazole	45153	52.11	306.9280	0.492
6	Insecticide	Isolan	33018	82.63	210.1269	3.090
7	Herbicide	3,4-Dichloraniline	25708	58.92	159.9724	0.442
8	Acaricide	Aminocarb	12990	86.00	267.1367	7.937
9	Herbicide	Simazine	11075	50.16	260.0936	6.489
10	Insecticide	Tebufenozide	9778	74.50	351.2084	7.937
11	Pesticide	2,4-Dinitrophenol	9074	81.94	183.0040	6.938
12	Insecticide	Tebufenozide	7814	78.44	397.2119	7.338
13	Herbicide	Atrazine	7211	51.02	260.0934	6.489
14	Herbicide	Chlorphenprop-methyl	6962	80.16	231.0006	5.689
15	Herbicide	Cycluron	5913	75.18	243.1701	4.939
16	Insecticide	Demeton-O	5572	65.03	257.0431	3.090
17	Pesticide	4,4'-Bipyridyl	5267	83.69	215.0828	3.090
18	Herbicide	Sultallat (CDEC)	3921	58.78	268.0234	4.190
19	Herbicide	Diethofencarb	3777	52.53	266.1404	6.988
20	Herbicide	Fluometuron	3759	74.71	231.0764	3.040
21	Fungicide	Triamiphos	3641	59.10	293.1302	6.888
22	Insecticide	DNOC (2,4-Dinitro-o-kresol)	3399	76.77	257.0430	3.090
23	Herbicide	Bentranil	3197	75.92	268.0593	5.339
24	Insecticide	Monocrotophos	3197	69.35	268.0593	5.339
25	Insecticide	Carbosulfan	3178	66.86	379.2045	7.838
26	Insecticide	Ethoprofos	3162	50.66	241.0510	3.140
27	Insecticide	Pirimicarb	2962	61.12	283.1414	3.290
28	Insecticide	Diafenthiuron	2891	60.43	383.2194	6.838
29	Pesticide	Ancymidol	2887	60.08	255.1111	6.888
30	Fungicide	Furmecyclox	2870	51.92	250.1441	6.938
31	Herbicide	Fluroxypyr	2769	63.80	252.9605	4.789
32	Insecticide	Mephosfolan	2717	61.70	268.0240	4.140
33	Fungicide	Pyrifenox	2654	50.87	293.0260	4.240

Nr	Class	Name	Abundance	Score	m/z	RT (min)
34	Fungicide	2,3-Dichlorophenol	2593	58.61	206.9636	0.343
35	Herbicide	Metoxuron	2486	67.21	287.0816	6.589
36	Insecticide	Crufomat	2388	52.95	336.0783	6.838
37	Herbicide	Cyanazine	2335	57.02	239.0825	3.090
38	Insecticide	Flufenoxuron	2225	56.53	533.0354	5.539
39	Herbicide	Oxasulfuron	2184	80.71	405.0852	4.589
40	Insecticide	Deguelin	2148	52.21	393.1398	3.340
41	Fungicide	Dimoxystrobin	2138	52.11	385.1780	3.140
42	Herbicide	Deiquat	2068	82.22	243.1133	3.190
43	Insecticide	Dimetilan	1878	75.14	285.1216	3.140
44	Herbicide	Pyridate	1853	53.48	377.1091	3.240
45	Acaricide	Aminocarb	1824	61.25	267.1367	7.188
46	Herbicide	Oryzalin	1754	52.22	345.0853	3.490
47	Fungicide	Hydrargaphen	1530	54.97	981.0341	0.542
48	Insecticide	Trimethacarb	1511	56.45	252.1252	7.438
49	Plant growth regulator	Dikegulac	1367	52.57	333.1217	3.190
50	Insecticide	Fenazaquin	1326	60.83	365.1871	7.838
51	Herbicide	Carbetamide	1268	71.71	295.1312	4.440
52	Fungicide	Fuberidazole	1211	71.74	183.0569	3.090
53	Fungicide	Pencycuron	1200	55.24	387.1486	3.190
54	Acaricide	Dinobuton	1167	62.06	385.1248	7.987
55	Fungicide	Ethylendibromide	1082	52.40	232.8645	8.337
Maize 15						
1	Insecticide	Tebufenozide	78161	72.24	351.2092	6.841
2	Herbicide	Dinoseb-acetate	55912	86.32	341.0970	0.495
3	Herbicide	Medinoterbacetate	55912	86.32	341.0970	0.495
4	Insecticide	Tebufenozide	11688	79.99	351.2090	7.940
5	Herbicide	3,4-Dichloraniline	10567	57.71	159.9724	0.495
6	Insecticide	Tebufenozide	9281	78.79	397.2146	7.341
7	Acaricide	Aminocarb	7721	81.33	267.1371	7.940
8	Fungicide	Furmecyclox	5842	54.71	250.1457	6.941
9	Insecticide	Cevadin	5055	59.22	636.3342	3.243
10	Insecticide	DNOC (2,4-Dinitro-o-kresol)	4960	94.93	257.0420	3.093
11	Pesticide	2,4-Dinitrophenol	4746	84.70	183.0043	7.391
12	Insecticide	Demeton-O	4259	53.69	257.0429	3.093
13	Insecticide	Gossypol	4159	71.65	577.2088	7.341
14	Insecticide	Carbosulfan	3550	73.49	379.2061	6.891
15	Herbicide	Hexazinone	3407	76.59	251.1515	6.991
16	Acaricide	Aminocarb	3118	75.18	267.1367	7.041
17	Herbicide	Cycluron	2911	55.00	243.1697	4.942
18	Fungicide	Flusilazol	2565	78.01	314.0957	3.793
19	Insecticide	Methoxyfenozide	1830	65.30	427.2223	7.141
20	Pesticide	4,4'-Bipyridyl	1809	80.35	215.0829	3.143

Nr	Class	Name	Abundance	Score	m/z	RT (min)
21	Insecticide	Benfuracarb	1739	51.72	455.1870	7.241
22	Insecticide	Fenpropathrin	1571	50.17	394.1641	6.841
23	Herbicide	Bromofenoxim	1451	51.66	519.8819	0.445
24	Insecticide	Cartap	1370	73.24	296.0748	6.891
25	Herbicide	Diethofencarb	1211	53.06	266.1405	3.343
26	Insecticide	Thiofanox	1025	59.76	217.0999	4.093
Pecan 1						
1	Fungicide	Anilazine	210749	57.93	272.9489	0.447
2	Insecticide	Cevadin	125594	76.49	636.3368	3.245
3	Herbicide	Medinoterbacetate	67848	90.97	341.0975	0.697
4	Herbicide	Dinoseb-acetate	62475	90.96	341.0975	0.697
5	Insecticide	Tebufenozide	35345	76.37	351.2092	6.843
6	Fungicide	Etridiazole	15224	50.78	306.9279	0.447
7	Insecticide	DNOC (2,4-Dinitro-o-kresol)	11072	65.16	257.0408	3.045
8	Fungicide	Triamiphos	9120	53.44	293.1316	6.893
9	Herbicide	Diethofencarb	9087	51.25	266.1414	7.093
10	Insecticide	Demeton-O	8368	55.72	257.0411	3.095
11	Herbicide	Bromofenoxim	6756	55.24	519.8819	0.447
12	Pesticide	4,4'-Bipyridyl	5555	76.11	215.0837	3.145
13	Insecticide	Tebufenozide	5145	91.95	397.2147	7.292
14	Insecticide	Fenazaquin	5064	56.72	351.1741	7.342
15	Insecticide	Etrimfos	4890	64.87	291.0591	6.843
16	Acaricide	Aminocarb	4120	70.99	267.1369	7.143
17	Herbicide	Cycluron	3997	71.71	243.1700	4.944
18	Pesticide	2,4-Dinitrophenol	3567	79.70	183.0046	7.892
19	Acaricide	Phenkapton	3262	62.52	374.9289	7.093
20	Fungicide	Urbazid	3262	66.08	374.9289	7.093
21	Insecticide	Fenpropathrin	3096	72.57	394.1636	7.892
22	Herbicide	Carbetamide	2994	68.16	295.1295	2.995
23	Insecticide	Carbosulfan	2916	64.81	379.2048	7.842
24	Herbicide	Difenoxuron	2815	70.46	285.1247	6.943
25	Insecticide	Flufenoxuron	2801	55.41	533.0357	6.943
26	Herbicide	Bentazone	2788	80.30	239.0491	3.045
27	Herbicide	Oxasulfuron	2776	58.61	405.0849	4.644
28	Herbicide	Aziprotryne	2555	61.72	284.0947	6.893
29	Pesticide	Ancymidol	2341	53.50	255.1113	6.893
30	Herbicide	Cyanazine	2150	57.48	239.0825	3.095
31	Fungicide	Cyprodinil	1864	75.28	284.1395	7.892
32	Herbicide	Defenurone	1751	62.41	195.0779	6.943
33	Acaricide	Aminocarb	1633	65.67	267.1337	3.195
34	Herbicide	Deiquat	1574	84.19	229.0983	3.345
35	herbicide	Mefenacet	1562	62.98	297.0691	6.893
36	Fungicide	Pencycuron	1561	78.10	327.1263	3.095
37	Herbicide	Fluometuron	1548	70.79	231.0757	3.045

Nr	Class	Name	Abundance	Score	m/z	RT (min)
38	Insecticide	Dimetilan	1543	64.30	285.1213	3.095
39	Fungicide	Benomyl	1376	54.60	335.1382	3.445
40	Herbicide	Neburon	1293	54.28	319.0644	3.095
41	Insecticide	Clenpirin	1273	58.29	343.1007	3.345
42	Insecticide	Nitenpyram	1253	58.64	315.0872	3.095
43	Herbicide	Morfamquate	1244	71.20	467.2652	7.892
44	Insecticide	Pyrethrin I	1203	69.43	373.2048	6.843
45	Herbicide	Phenalenone	1160	64.85	179.0478	0.547
46	Herbicide	Prodiamine	1140	59.27	349.1153	6.993
47	Fungicide	Fuberidazole	1129	55.22	183.0580	3.095
48	Fungicide	Cymoxanil (Curzate)	1114	62.08	257.0914	3.095
49	Insecticide	Tebupirimfos	1098	52.22	317.1074	3.045
Pecan 2						
1	Insecticide	Cevadin	139740	78.27	636.3370	3.235
2	Herbicide	Diflufenican	100112	90.71	439.0723	0.587
3	Herbicide	Dinoseb-acetate	87234	91.57	341.0977	0.487
4	Herbicide	Medinoterbacetate	87234	91.57	341.0977	0.487
5	Fungicide	Anilazine	73006	51.79	272.9490	0.437
6	Acaricide	Aminocarb	18784	83.80	267.1371	7.932
7	Herbicide	3,4-Dichloraniline	16065	57.55	159.9726	0.437
8	Herbicide	Bensulfuron-methyl	8879	51.91	455.0848	0.487
9	Insecticide	Tebufenozide	8728	85.62	397.2138	7.333
10	Insecticide	Tebufenozide	6026	76.93	351.2091	7.932
11	Pesticide	Isocarbophos	5361	61.35	288.0493	6.933
12	Herbicide	Imazapyr	5297	80.56	306.1087	0.687
13	Herbicide	Diethofencarb	4666	53.60	266.1411	7.083
14	Insecticide	Allethrin II	4555	58.97	405.1947	7.183
15	Insecticide	5-Hydroxyimidacloprid	4373	58.29	330.0597	5.034
16	Pesticide	2,4-Dinitrophenol	4373	76.54	183.0043	7.333
17	Fungicide	Ziram	4298	55.48	362.9290	0.487
18	Insecticide	Dicrotophos Bildrin	3718	68.65	296.0896	1.186
19	Pesticide	4,4'-Bipyridyl	3578	76.94	215.0826	3.135
20	Insecticide	Isolan	3572	65.88	210.1273	3.135
21	Fungicide	Furmecyclox	3400	50.08	250.1459	6.933
22	Insecticide	Monocrotophos	3162	62.91	268.0600	5.384
23	Insecticide	Carbosulfan	3074	66.24	379.2043	7.882
24	Herbicide	Primisulfuron-methyl	3018	51.77	513.0326	0.487
25	Insecticide	Bromfenvinphos-methyl	2863	56.63	420.8832	0.437
26	Herbicide	Falone	2590	65.39	706.9329	7.183
27	Pesticide	Ancymidol	2435	69.77	255.1132	3.185
28	Herbicide	Fluometuron	2395	69.79	231.0744	3.035
29	Insecticide	Pirimicarb	2349	73.93	283.1408	3.685
30	Insecticide	Tebufenozide	2259	60.70	351.2057	6.883
31	Pesticide	Butocarboxim-sulphoxide	2197	85.47	251.0689	0.687

Nr	Class	Name	Abundance	Score	m/z	RT (min)
32	Herbicide	Dinoseb	2189	57.83	299.0867	3.385
33	Insecticide	Fenazaquin	2151	81.28	365.1879	6.933
34	Herbicide	Sultallat (CDEC)	2008	51.38	282.0402	3.735
35	Plant growth regulator	Thidiazuron	1999	52.28	279.0586	6.783
36	Fungicide	Cyprodinil	1989	72.88	284.1403	7.882
37	Herbicide	Daminozide	1989	72.60	219.0977	1.036
38	Insecticide	Carbaryl	1967	52.26	260.0944	6.583
39	Herbicide	Chloridazone	1935	58.22	220.0268	3.435
40	Herbicide	Deiquat	1869	85.41	243.1138	3.135
41	Herbicide	Propyzamide	1716	55.55	314.0332	5.084
42	Fungicide	Phenylchlorophenol	1589	58.94	249.0320	6.833
43	Herbicide	Oxasulfuron	1488	56.60	405.0870	4.634
44	Acaricide	Dinobuton	1462	59.04	385.1245	3.635
45	Insecticide	Dimetilan	1457	74.57	285.1207	3.185
46	Insecticide	Cyanthoate	1451	66.47	353.0932	3.984
47	Pesticide	Methiocarb-sulfoxide	1351	69.87	286.0749	3.335
48	Insecticide	Methoxyfenozide	1349	55.59	367.2019	6.833
49	Herbicide	Bentazone	1314	68.89	299.0722	4.434
50	Fungicide	Cymoxanil (Curzate)	1202	78.13	243.0721	3.135
51	Herbicide	Fenaclon	1199	54.85	270.0919	3.135
52	Fungicide	Benomyl	1187	66.45	335.1377	6.933
53	Herbicide	Metoxuron	1100	59.01	227.0581	0.687
54	Insecticide	Bulan	1047	52.50	322.0398	0.637
55	Pesticide	Pyridaben	1040	61.40	423.1535	4.134
56	Insecticide	Mephosfolan	1021	60.05	268.0244	4.184
57	Herbicide	Terbacil	1012	57.91	261.0646	3.235
Pecan 3						
1	Fungicide	Anilazine	169814	50.99	272.9489	0.449
2	Insecticide	Cevadin	150463	73.86	636.3368	3.247
3	Fungicide	Etridiazole	69834	57.65	306.9288	0.499
4	Insecticide	Tebufenozide	11690	73.51	351.2094	7.944
5	Pesticide	2,4-Dinitrophenol	10845	72.73	183.0044	7.394
6	Insecticide	DNOC (2,4-Dinitro-o-kresol)	9995	85.95	257.0413	3.097
7	Insecticide	Tebufenozide	9960	57.51	351.2065	6.845
8	Herbicide	Bromofenoxim	8908	58.07	519.8830	0.449
9	Insecticide	Demeton-O	8534	72.47	257.0412	3.097
10	Insecticide	Tebufenozide	8071	61.42	397.2155	7.294
11	Herbicide	Dinoseb-acetate	6744	90.35	341.0977	0.699
12	Herbicide	Medinoterbacetate	6296	90.15	341.0977	0.699
13	Herbicide	Falone	4789	64.32	708.9311	7.195
14	Fungicide	Folpet	4675	59.14	341.8978	0.499
15	Insecticide	Disulfoton	4246	58.41	273.0238	1.848
16	Fungicide	Furmecyclox	4177	51.81	250.1462	6.945

Nr	Class	Name	Abundance	Score	m/z	RT (min)
17	Herbicide	Dinoseb	4089	54.00	285.0707	3.347
18	Herbicide	Cyprazine	3997	58.85	286.1100	7.294
19	Acaricide	Aminocarb	3474	63.37	267.1373	7.045
20	Insecticide	Carbosulfan	3386	71.52	379.2050	7.844
21	Herbicide	Cycluron	3238	79.50	243.1703	4.946
22	Herbicide	Dinoseb	2775	57.27	299.0862	3.696
23	Pesticide	4,4'-Bipyridyl	2547	80.77	215.0839	3.147
24	Insecticide	Nitenpyram	2087	50.97	269.0789	6.945
25	Herbicide	Carbetamide	1940	52.16	295.1269	2.997
26	Pesticide	Cadusafos	1808	54.65	269.0791	6.945
27	Insecticide	Cartap	1667	56.78	296.0757	6.945
28	Pesticide	Ancymidol	1451	76.48	255.1131	3.147
29	Insecticide	Mevinphos	1437	75.84	283.0580	3.197
30	Insecticide	Aldicarb	1388	68.78	189.0696	3.147
31	Insecticide	Thiofanox	1387	75.97	217.0996	3.197
32	Insecticide	Diafenthiuron	1337	56.19	383.2171	6.895
33	Herbicide	Cyanazine	1312	50.47	239.0824	3.197
34	Acaricide	Dinobuton	1305	57.80	385.1281	7.994
35	Herbicide	Morfamquate	1181	65.96	527.2894	7.544
36	Plant growth regulator	Dikegulac	1121	66.66	333.1227	7.944
37	Pesticide	4,4'-Bipyridyl	1117	78.42	215.0817	6.845
38	Herbicide	Fluometuron	1088	56.25	231.0780	3.047
39	Herbicide	MCPA-thioethyl	1068	55.14	243.0233	1.698
40	Herbicide	Deiquat	1022	84.99	229.0981	3.147
Pecan 4						
1	Herbicide	Diethofencarb	215922	58.28	266.1420	7.938
2	Insecticide	Cevadin	120786	76.56	636.3367	3.241
3	Fungicide	Anilazine	74169	54.10	272.9493	0.443
4	Fungicide	Etridiazole	45526	51.91	306.9280	0.493
5	Herbicide	Dinoseb-acetate	30785	93.54	341.0977	0.693
6	Herbicide	Medinoterbacetate	30785	93.54	341.0977	0.693
7	Insecticide	DNOC (2,4-Dinitro-o-kresol)	17967	88.98	257.0407	3.091
8	Insecticide	Tebufenozide	11766	72.18	351.2067	6.839
9	Herbicide	Diethofencarb	8002	51.49	266.1413	7.089
10	Pesticide	Aldicarb-sulfone (Aldoxycarb)	7598	83.45	267.0635	0.693
11	Herbicide	Dinoseb	6744	62.26	285.0705	3.491
12	Insecticide	Tebufenozide	6017	75.14	397.2149	7.288
13	Herbicide	Imazapyr	5652	83.56	306.1087	0.693
14	herbicide	MCPA-thioethyl	4111	68.71	243.0242	2.291
15	Pesticide	2,4-Dinitrophenol	3910	80.16	183.0047	7.888
16	Pesticide	Butocarboxim-sulphoxide	3559	77.76	251.0690	0.693
17	Herbicide	Chlorphenprop-methyl	3017	71.90	231.0009	5.740
18	Herbicide	Orbencarb	2943	50.22	256.0566	0.943

Nr	Class	Name	Abundance	Score	m/z	RT (min)
19	Insecticide	Phorate	2578	51.79	259.0040	2.341
20	Pesticide	4,4'-Bipyridyl	2544	86.79	215.0828	3.141
21	Fungicide	Furmecyclox	2526	58.54	250.1447	6.939
22	Insecticide	Aldicarb	2474	59.75	189.0695	3.141
23	Fungicide	Triamiphos	2248	58.93	293.1304	6.889
24	Insecticide	Carbosulfan	1974	73.52	379.2066	7.838
25	Pesticide	Ancymidol	1680	76.22	255.1122	3.241
26	Herbicide	Deiquat	1660	85.31	243.1138	3.341
27	Fungicide	Iprobenfos	1643	61.68	347.1090	3.091
28	Herbicide	Aziprotryne	1631	52.72	270.0767	6.939
29	Insecticide	Prothoate	1476	51.42	284.0552	6.839
30	Insecticide	Tebufenozide	1455	74.53	351.2072	7.938
31	Herbicide	Glyphosate	1444	63.51	168.0082	2.991
32	Insecticide	MNFA	1365	55.74	262.0873	6.889
33	Fungicide	Hexaconazole	1363	57.42	312.0693	6.789
34	Insecticide	Dicrotophos Bildrin	1344	50.81	282.0741	0.693
35	Insecticide	Monocrotophos	1299	57.95	282.0738	0.693
36	Fungicide	Pencycuron	1237	50.62	327.1284	3.291
37	Insecticide	Thiofanox	1198	63.84	217.1005	3.141
38	Insecticide	Bomyl	1180	59.68	281.0433	0.693
39	Insecticide	Pirimicarb	1112	71.33	283.1431	4.040
40	Fungicide	Procymidone	1063	55.75	328.0168	4.140
41	Herbicide	Phenalenone	1055	72.80	179.0485	0.643
42	Fungicide	Hydrargaphen	1050	55.32	981.0420	0.543
43	Fungicide	Famoxadone	1009	73.49	373.1209	3.940
44	Acaricide	Dinobuton	1003	54.40	385.1270	3.341
45	Fungicide	Benomyl	1001	72.25	335.1369	3.191

ANNEXURE C: TITLE PAGE OF PUBLISHED MANUSCRIPT

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Review

Agrochemicals in freshwater systems and their potential as endocrine disrupting chemicals: A South African context☆

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ABSTRACT

South Africa is the largest agrochemical user in sub-Saharan Africa, with over 3000 registered pesticide products. Although they reduce crop losses, these chemicals reach non-target aquatic environments via leaching, spray drift or run-off. In this review, attention is paid to legacy and current-use pesticides reported in literature for the freshwater environment of South Africa and to the extent these are linked to endocrine disruption. Although banned, residues of many legacy organochlorine pesticides (endosulfan and dichlorodiphenyltrichloroethane (DDT)) are still detected in South African watercourses and wildlife. Several current-use pesticides (triazine herbicides, glyphosate-based herbicides, 2,4-dichlorophenoxyacetic acid (2,4-D) and chlorpyrifos) have also been reported. Agrochemicals can interfere with normal hormone function of non-target organism leading to various endocrine disrupting (ED) effects: intersex, reduced spermatogenesis, asymmetric urogenital papillae, testicular lesions and infertile eggs. Although studies investigating the occurrence of agrochemicals and/or ED effects in freshwater aquatic environments in South Africa have increased, few studies determined both the levels of agricultural pesticides present and associated ED effects. The majority of studies conducted are either laboratory-based employing *in vitro* or *in vivo* bioassays to determine ED effects of agrochemicals or studies that investigate environmental concentrations of pesticides. However, a combined approach of bioassays and chemical screening will provide a more comprehensive overview of agrochemical pollution of water systems in South Africa and the risks associated with long-term chronic exposure.

1. Introduction

South Africa's semi-arid climate makes it ideal for the cultivation of various crops (Quinn et al., 2011; Yahaya et al., 2017), including maize, soybean, sunflower, wheat, sugar cane and a variety of citrus fruits. Approximately 15 million hectares of South Africa's land is used for cultivation, accounting for 12–13% of the country's total area of 122 million hectares (Van der Laan et al., 2017). South Africa has an estimated poverty rate of 28%, which is equivalent to more than 16 million individuals living in extreme poverty (World Poverty Clock, 2020). As a result, the country's emerging economy is highly dependent on agriculture in terms of job creation and poverty alleviation (Olujimi et al., 2010). However, crop production in South Africa is not considered sustainable because it comes at an ecological cost due to the vast amount of agrochemicals applied (Van der Laan et al., 2017; Yahaya et al., 2017). In the 1970s, Africa's pesticide load significantly increased when large quantities of dichlorodiphenyltrichloroethane (DDT), malathion and many other pesticides were "donated" to African countries for malaria vector control, following their ban in developed countries (Osibanjo et al., 2002; Manyilizu, 2019). Apart from disease vector control, the strive for food security has caused increased use of agrochemicals to reduce crop losses associated with a variety of pests (Archer et al., 2020). To date, South Africa is the largest pesticide user in sub-Saharan Africa (Ansara-Ross et al., 2012), with over 3000 pesticide products registered for use (Van der Laan et al., 2017), even though this is not internationally recognised: South Africa did not even feature in a publication by Sharma et al. (2019) who reviewed global pesticide usage and its effect on ecosystems.

South Africa's subtropical location, warm temperate conditions and variable rainfall (~464 mm annually) makes the country especially vulnerable in terms of water availability (South African

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