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Genetically modified glyphosate tolerant maize, soybean and oilseed rape versus conventionally grown varieties – agricultural practices, residues of glyphosate, other pesticides and metabolites, and implications for toxicity

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Competence of VKM experts

Persons working for VKM, either as appointed members of the Committee or as external experts, do this by virtue of their scientific expertise, not as representatives for their employers or third party interests. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

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Summary

The adoption worldwide of genetically modified (GM) crops has been rapid since they were introduced in USA in 1994. Globally, close to 190 million hectares (ha) of GM crops were planted in 2017. At present, only one GM crop/plant (insect resistant maize MON 810) is grown in the EU. Many GM plants are approved in EU for import and for use in food or feed. However, the majority is used in feed. In Norway, no GM crops are approved for cultivation or import for use in food or feed.

The primary introduced trait in GM crops is herbicide tolerance (HT), of which tolerance to the broad-spectrum herbicide glyphosate is by far predominant.

The large scale and continued shift from farming conventional crops towards adoption of GMHT crops has had a significant impact on agricultural practices, e.g. reduced tillage, which may improve soil quality and reduce soil erosion, and weed management strategies, including changes in herbicide treatment of crops. This could potentially affect the composition and levels of total herbicide residues in the GMHT crops and subsequently affect the health of consumers or farmed animals through food and feed.

Information on residue levels of herbicide(s) that GMHT crops are modified to tolerate, and their metabolites, are not included in the documentation provided by the developers of GMHT crops when applying for authorisation in the EU, since this information is not mandatory for these applications. Such data are instead supplied to the bodies that assess plant protection products and sets maximum residue levels (European Food Safety Authority, EFSA, Panel on Plant Protection Products and their Residues, PPR).

The Norwegian Scientific Committee for Food and Environment (VKM) has repeatedly reported this lack of information as a data gap in risk assessments of GMHT crops, and therefore initiated this project. Three crops were chosen for scrutiny in the project: maize, soybean and oilseed rape. Maize and soybean are the two major GM food and feed crops in the world, and oilseed rape was included since it is grown as a conventional crop in Norway.

The terms of reference (ToR) with answers are described below:

ToR 1. A comparison between common weed control practices used with glyphosate tolerant crops and those used with conventional crops

The effect of the increasing adoption of GMHT crops is reflected in the user statistics of herbicides in USA, where such crops have become dominant. The most obvious trend in use of herbicides is the extensive increase in use of glyphosate. On a volume basis glyphosate contributed less than 5% of total herbicides used in maize and soybean cultivation in the early 1990's. In 2015, glyphosate constituted 76% of herbicides applied in soybeans and 37% in maize. Globally, the use of glyphosate increased almost 15-fold in 20 years, to reach

826 000 tons in 2014. In some countries in South America, there has been an approximately five-fold increase in the area load of herbicides after the introduction of GM crops.

In conventional crops, broad-spectrum herbicides such as glyphosate are mainly used for burndown of weeds after harvest and/or before planting. In glyphosate tolerant crops, glyphosate can also be applied to control weeds after emergence of the crops, which reduces the need for use of other, more selective herbicides. The differences in patterns of herbicide use between conventional and GMHT crops may have effects on the level and composition of residues of herbicides in the harvested crops.

The area loads of herbicides other than glyphosate used on soybean and maize initially declined after introduction of glyphosate tolerant varieties. However, several studies have shown an increased use of non-glyphosate herbicides in glyphosate tolerant crops in recent years. An example is that adopters of glyphosate tolerant maize went from using 1.31 kg/ha less than before of non-glyphosate herbicides in 1998, to only 0.32 kg/ha less in 2011. This has been linked to the development of glyphosate resistant weeds, requiring farmers to use herbicides other than glyphosate.

ToR 2. Residue levels of glyphosate, its metabolites and other relevant herbicides used on glyphosate tolerant and conventional crops

There is very little data available in the open literature on glyphosate residue levels in conventional and glyphosate tolerant maize, soybean and oilseed rape. The data collected from our literature searches were therefore insufficient for a systematic comparison of residue levels between conventional and glyphosate tolerant maize, soybean and oilseed rape. Fourteen research papers were found to include glyphosate residue levels, some also including metabolites. However, the data were very heterogeneous and practically not comparable. Residues were measured in different parts of the plants (leaf, stem, seed etc.), application rates varied, as did seasonal treatment and sampling times.

To answer ToR 2, also residues of other relevant herbicides were to be compared between glyphosate tolerant crops and conventional crops. For this purpose, the project group sought to compare residue levels of 10 selected herbicides in maize and soybean. The 10 herbicides were selected based on their total annual use in USA (in tons) according to surveys conducted in 2015 and 2016 for maize and soybean, respectively. However, the literature searches returned no relevant publications. In addition, since the available survey and monitoring data did not distinguish between GM and conventionally grown crops, it was not possible to conclude whether these two varieties differed regarding residue levels of the 10 selected herbicides in maize and soybeans.

ToR 3. A description of how the genetic modification(s) used to make a plant tolerant against glyphosate may influence the metabolism of glyphosate or other plant protection products, and

ToR 4. An evaluation of whether possible changes in the spectrum of metabolites may have implications for the toxicity of glyphosate tolerant crops

Literature searches, performed to investigate whether or not genetic modifications introduced to make a plant tolerant against glyphosate may influence the metabolism of glyphosate or other plant protection products, returned insufficient relevant data to answer ToR 3. Out of the three main genetic modifications used to make plants tolerant to glyphosate, only the introduction of a gene expressing the enzyme glyphosate *N*-acetyl transferase (GAT) results in new metabolites of glyphosate: *N*-acetyl-glyphosate and *N*-acetyl-AMPA (in addition to the degradation product of glyphosate (AMPA)). It was concluded in several risk assessments both by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) and EFSA that the toxicological profile of glyphosate would also cover these metabolites, and the health based guidance value (acceptable daily intake, ADI) are derived for the sum of glyphosate and the three metabolites. Therefore, these metabolites were not considered likely to have implications for the toxicity of GAT-expressing maize, soybean and oilseed rape.

No relevant publications were found when either of the two enzymes glyphosate oxidoreductase (GOX) or GAT was combined with any of 15 selected herbicides, fungicides and insecticides. The 15 pesticides were chosen based on available data on their highest annual use in USA (in tons) for maize and soybean in the period 2012 – 2016. Three publications were identified describing the GAT enzyme, its specificity towards glyphosate as substrate and affinity to relevant chemical groups. Based on structure-activity studies and expert judgement, it was considered unlikely that any of the 15 chosen pesticides would function as substrate for the GAT enzyme. Thus, it was unlikely that this enzyme would affect the metabolism and therefore the toxicity of these 15 pesticides. However, experimental data is needed to answer these ToRs with more certainty.

Key words: conventional crops, genetically modified crops, herbicide residues, maize, oilseed rape, soybean, glyphosate, pesticides, Norwegian Scientific Committee for Food and Environment

Sammendrag

Bruken av genmodifiserte (GM) planter har økt i raskt tempo siden de ble introdusert i USA i 1994. Globalt ble det dyrket GM-planter på nærmere 190 millioner hektar (ha), i 2017. Per i dag er det kun MON 810, en insektresistent mais, som er godkjent for dyrking i EØS-området. Mange GM-planter har imidlertid blitt godkjent for import til EU til bruk i mat og fôr. Majoriteten av disse brukes kun til fôr. I Norge er ingen GM-planter godkjent hverken til dyrking eller for import til bruk i mat eller fôr.

Den hyppigst introduserte egenskapen i GM-planter er økt toleranse for ugressmidler (herbicidtoleranse, HT), og da i all hovedsak toleranse for det bredspektrede ugressmiddelet glyfosat.

Overgang fra dyrking av konvensjonelle planter til dyrking av genmodifiserte planter som er tolerante for ugressmidler (GMHT), har betydelig innvirkning på landbrukspraksis. Eksempler er mulighet for redusert jordarbeiding som kan gi bedre jordkvalitet og redusere erosjon, endrede strategier for bekjempelse av ugress inkludert endringer i sprøytepraksis. Dette kan potensielt påvirke sammensetningen og totalnivået av plantevernmiddelrester i plantene, og følgelig ha betydning for helsen til forbrukere og husdyr (via inntak av mat og fôr). Ved søknad om godkjenning av GMHT-planter i EU stilles det ikke krav til dokumentasjon om restnivåer av ugressmidlene de er utviklet for å tolerere. Det samme gjelder for restnivåer av nedbrytningsprodukter av ugressmidlene. Denne informasjonen oppgis i stedet til ekspertpanelet på plantevernmidler og plantevernmiddelrester i den europeiske myndighet for næringsmiddeltrygghet, EFSA, som vurderer søknader om godkjenning av plantevernmidler, og fastsetter maksimumsgrenser for tolererte restnivåer.

Vitenskapskomiteen for mat og miljø (VKM) har gjentatte ganger påpekt at mangel på slik dokumentasjon er et kunnskapshull ved risikovurdering av slike planter, og tok derfor initiativ til dette prosjektet. For å besvare problemstillingene valgte prosjektgruppen å ta utgangspunkt i mais, soya og oljeraps. Mais og soya er de mest dyrkede matplantene innen produksjon av genmodifisert mat og fôr, og oljeraps dyrkes konvensjonelt i Norge.

Problemstillinger (Terms of Reference, heretter kalt «ToR») med svar er angitt nedenfor:

ToR 1. En sammenligning mellom vanlige metoder for ugressbekjempelse som brukes ved dyrking av glyfosattolerante planter og de metodene som brukes ved dyrking av konvensjonelle planter

Følgene av overgang til bruk av genmodifiserte planter som er tolerante for ugressmidler, gjenspeiles i bruksstatistikken for ugressmidler i USA, hvor slike planter har blitt dominerende. Den mest åpenbare trenden er den omfattende økningen i bruk av glyfosat. Beregnet ut fra volum utgjorde glyfosat tidlig på 1990-tallet mindre enn 5 prosent av totalmengden av ugressmidler brukt til dyrkning av mais og soya. I 2015 utgjorde glyfosat 76 prosent av alle ugressmidler brukt på soya, og 37 prosent for mais. Globalt økte bruken av glyfosat nesten 15 ganger i løpet av 20 år, til 826 000 tonn i 2014. I enkelte land i Sør-Amerika har det vært en nær fem ganger økning i mengden per areal over tid ('area load') av brukte ugressmidler etter at GM-planter ble introdusert.

Ved dyrking av konvensjonelle planter brukes bredspektrede ugressmidler som glyfosat hovedsakelig til å svi av ugress etter høsting og/eller før såing, fordi glyfosat ellers ville drepe selve nytteplanten. Med glyfosattolerante planter kan glyfosat i tillegg brukes til å kontrollere ugress etter fremvekst av nytteplanten, noe som reduserer behovet for bruk av andre mer selektive ugressmidler. Disse forskjellene i bruk av ugressmidler mellom konvensjonelle planter og GMHT-planter kan påvirke nivåer og sammensetning av plantevernmiddelrester i avlinger.

I den første perioden etter at glyfosattolerante planter kom på markedet, gikk bruken av andre/selektive ugressmidler på soya og mais ned. De senere årene har imidlertid flere studier vist at andre ugressmidler i økende grad også brukes på glyfosattolerante planter. For eksempel viser tall fra 1998 at bønder som hadde gått over til å dyrke glyfosattolerant mais brukte 1,31 kg/ha mindre av andre ugressmidler enn tidligere. I 2011 var dette tallet kun 0,32. Økningen har blitt koblet til utviklingen av glyfosatresistente ugress, som har tvunget bønder til å bruke andre ugressmidler enn glyfosat.

ToR 2. Data på restnivåer av glyfosat og dets metabolitter, samt andre relevante ugressmidler brukt både på glyfosattolerante planter og på konvensjonelle planter

I tilgjengelig litteratur var det svært lite data å finne på glyfosatrester, både for konvensjonelle og for glyfosattolerante sorter av mais, soya og oljeraps. Prosjektgruppen fant 14 vitenskapelige artikler som inneholdt data på målinger av glyfosatrester, hvorav enkelte også inkluderte målinger av metabolitter. Dataene var imidlertid svært heterogene og i praksis ikke sammenlignbare. Restmengder var målt i ulike deler av plantene (blad, stilk, frø osv.), det ble brukt forskjellige konsentrasjoner av glyfosat, samt ulike tidspunkter for sprøytning og prøvetaking. Dataene fra våre litteratursøk var derfor utilstrekkelige for en systematisk sammenligning av restnivåene i konvensjonelle og glyfosattolerante avlinger.

Litteratursøkene ga ingen relevante treff for å kunne sammenligne restnivåer av 10 andre ugressmidler brukt på både konvensjonelle og glyfosattolerante sorter av mais og soya. Disse ugressmidlene ble valgt ut basert på årlig totalbruk i USA (antall tonn) i henholdsvis 2015 og 2016. Databasene og overvåkningsdataene differensierte ikke mellom konvensjonelle og genmodifiserte sorter. Det var derfor ikke mulig å konkludere hvorvidt det var noen forskjell i restmengder av de ti utvalgte ugressmidlene mellom konvensjonelle og glyfosattolerante sorter av mais og soya.

ToR 3. En beskrivelse av hvordan den/de genetiske modifikasjonen(e) som brukes til å gjøre en plante tolerant overfor glyfosat kan påvirke metabolismen av glyfosat og/eller andre plantevernmidler, og

ToR 4. En vurdering av om mulige endringer i spekteret av metabolitter kan ha implikasjoner for toksisiteten av glyfosattolerante planter

Litteratursøkene ga ingen relevante artikler på hvorvidt introduserte genmodifiseringer som gjør planter glyfosattolerante kan påvirke metabolismen av glyfosat eller andre plantevernmidler. Av de tre vanligste genetiske modifiseringene som brukes til å introdusere glyfosattoleranse hos planter, er det bare introduksjon av et gen som uttrykker enzymet glyfosat *N*-acetyl transferase (GAT), som fører til nye metabolitter av glyfosat: *N*-acetyl-glyfosat og *N*-acetyl-AMPA (i tillegg til metabolitten AMPA som dannes ved nedbrytning av glyfosat). 'The Joint FAO/WHO Meeting on Pesticide Residues' (JMPR) og EFSA har konkludert med at den toksikologiske profilen til glyfosat også dekker disse metabolittene, og den helsebaserte veiledende verdien (akseptabelt daglig inntak, ADI) er satt for summen av glyfosat og de tre metabolittene. Disse metabolittene ble derfor ansett å ikke ha betydning for toksisitet av avlinger av mais, soya eller raps som uttrykker GAT.

Det ble ikke funnet relevante publikasjoner som kombinerte søkeord for de to enzymene glyfosat oksidoreduktase (GOX) eller GAT, med ett eller flere av 15 utvalgte ugressmidler, soppmidler og insektmidler. De 15 plantevernmidlene ble valgt ut i fra høyeste årlige totalbruk i USA (antall tonn), for mais og soya, innrapportert i perioden 2012 – 2016. Tre publikasjoner beskrev GAT-enzymet og dets spesifisitet for glyfosat som substrat og affinitet for andre relevante kjemiske grupper. Basert på disse opplysningene, og en ekspertvurdering, ble det vurdert som lite sannsynlig at noen av de 15 utvalgte plantevernmidlene vil kunne fungere som substrat for GAT-enzymet. Det er dermed lite sannsynlig at GAT vil kunne påvirke metabolismen og toksisiteten til de 15 plantevernmidlene, men det er behov for eksperimentelle data for å kunne besvare ToR med større sikkerhet.

Abbreviations and glossary

Abbreviations

AMPA	-aminomethylphosphonic acid
AMS	-USDA Agricultural Marketing Service
APHIS	-USDA Animal and Plant Health Inspection Service
ARMS	-USDA Agricultural Resource Management Survey
EIQ	-environmental impact quotient
EFSA	-European Food Safety Authority
EPSPS	-5-enolpyruvylshikimate-3-phosphate synthase
ERS	-USDA Economic Research Service
FAO	-Food and Agriculture Organization of the United Nations
FDA	-United States Food and Drug Administration
GAP	-good agricultural practice
GAT	-glyphosate *N*-acetyl transferase
GM	-genetically modified
GMHT	-genetically modified herbicide-tolerant
GOX	-glyphosate oxidoreductase
HT	-herbicide tolerant
IPM	-integrated pest management
IR	-insect resistance
JPMR	-The Joint FAO/WHO Meeting on Pesticide Residues
LOD	-level (or limit) of detection
MRL	-maximum residue level
NASS	-USDA National Agricultural Statistics Service
NFSA	-Norwegian Food Safety Authority
PPPs	-plant protection products
ToR	-terms of reference
USDA	-United States Department of Agriculture
VKM	-Norwegian Scientific Committee for Food and Environment

Glossary

Acre	A measure of land area, where one acre is ~4047 m².
Acceptable daily intake (ADI)	A measure of the amount of a specific substance in food or drinking water that can be ingested (orally) on a daily basis over a lifetime without an appreciable health risk.
Application rates (of pesticides)	Defines the concentration of the pesticide active ingredient, and volume applied per area of a crop during treatment/spraying of the crop field.
Area load (AL) of pesticide(s)	An estimate of the total amount of applied pesticide(s) per hectare (ha) to the agricultural land where they have been used. Calculation of the area load of pesticide(s) for a given year/season depends on the amount of pesticide(s) sold, the properties of the pesticide(s) as well as the size of the area over which the product is estimated to have been applied.
Acute reference dose (ARfD)	Defined by the World Health Organization (WHO) as the amount of substance per kg of body weight that can be absorbed via the food with a meal or within a day without any perceptible risk to the consumer. It is only determined for substances which, owing to their acute toxicity, can cause health damage even on one-off or short-term exposure. As a rule, the ARfD value is derived from the lowest dose determined experimentally in animal experiments without a noticeable adverse effect (No Observed Adverse Effect Level, NOAEL), taking into account a safety factor of 100.
Broadcasting	In agriculture this term is used for a method of seeding that involves random scattering of seeds by hand, or mechanically, when planting a field.
Canola	The term 'canola' is derived from Canadian oil, low acid. Canola is a registered trademark of the Canadian Canola Association and refers to those varieties of *Brassica napus*, *B. rapa* and *B. juncacea* that meet specific standards on the levels of erucic acid and glucosinolates. Those varieties must yield oil low in erucic acid (below 2 %) and meal low in glucosinolates (total

	glucosinolates of 30 µmoles/g toasted oil free meal) (CODEX,1999), and are often referred to as "double low" varieties.
Conservation tillage	An agricultural management approach that aims to minimize the frequency or intensity of tillage operations in an effort to promote certain economic and environmental benefits.
Conventional crops	Crops having been genetically altered using a variety of traditional breeding methods, excluding biotechnology. Some breeding methods have been used for thousands of years, to develop traits, e.g. faster growth, higher yields, and pest and disease resistance.
Conventional plant breeding	Development or improvement of varieties using conservative tools for manipulating plant genomes within the natural genetic boundaries of the species. Plant breeders use methods and techniques that are based on the mode of reproduction of the species self-pollinating, cross-pollinating, or clonally propagated. The general strategy is to breed a variety whose genetic purity and productivity can be sustained by its natural mating system.
Crop rotation	The practice of growing a series of dissimilar or different types of crops in the same area in sequenced seasons. It helps in reducing soil erosion and increases soil fertility and crop yield.
Fungicide	A chemical or other substance used in agriculture to destroy or inhibit growth of fungi and fungal spores.
GM crops	Genetically modified crops, cultivated plants whose genetic characteristics have been altered by the insertion of one or several modified genes, or genes from another organism, using the techniques of genetic engineering.
Hectare	A hectare (ha) is a metric unit of surface area. Its use is widespread in agriculture where it is more practical than either square meters or square kilometres. 1 ha = 0.01 km^2, or 10000 m^2.
Herbicide	A chemical or other substance that is toxic to plants, used to destroy unwanted vegetation, e.g. weeds on agricultural land.

Insecticide	A chemical or other substance that is toxic to insects, e.g. used to control pests that infest cultivated plants in agriculture.
Integrated pest management (IPM)	An approach described by the UN's Food and Agriculture Organization (FAO) as integrated practices for economic control of pests. IPM consists of careful consideration of all available pest control techniques and subsequent integration of appropriate measures that discourage the development of pest populations and keep pesticides and other interventions to levels that are economically justified and reduce or minimise risks to human health and the environment.
Integrated weed management (IWM)	An approach described by the UN's Food and Agriculture Organization (FAO) that combines the use of complementary weed control methods such as grazing, herbicide application, land fallowing, and biological control to provide the best possible solutions to weed problems.
Maximum residue levels (MRL)	The upper levels of pesticide residues that are legally permissible in or on food or animal feed, based on good agricultural practice and the lowest consumer exposure necessary to protect vulnerable consumers.
Pest(s)	Loosely defined a pest is any living organism, whether animal, plant or fungus, which is invasive or troublesome to plants or animals, human or human concerns, livestock, or human structures.
Pesticide	A chemical or other substance used to control target pest(s), e.g. rodents, insects, weeds, and fungi.
Plant protection products	A chemical or other substance used to protect plants and plant products in agriculture, forestry and horticulture from attacks by fungi, pests and competing plants.
Tillage	The practice of working the soil to bring about better conditions for plant growth in agriculture.
Variety	The term is defined in different ways by different authors. The International Union for the Protection of New Varieties of Plants (UPOV)

	explains a plant variety with the following: “The term ‘species’ is a familiar unit of botanical classification within the plant kingdom. However, it is clear that within a species there can be a wide range of different types of plant. Farmers and growers need plants with particular characteristics and that are adapted to their environment and their cultivation practices. A plant variety represents a more precisely defined group of plants, selected from within a species, with a common set of characteristics.” For more information: https://www.upov.int/about/en/upov_system.html#what_is_a_pv
Volunteer (e.g. volunteer maize)	In agronomic terminology, a volunteer is a plant that grows on its own, rather than being deliberately planted by a farmer. E.g. in crop rotations, self-set plants from one year's crop may become established as weeds in the next crop of the rotation.

Background

The adoption worldwide of genetically modified (GM) crops has been rapid since they were introduced in USA in 1994. In 2017, the 21st year of commercialisation of biotech crops, 189.8 million hectares of GM crops were planted by ~17 million farmers in 24 countries (ISAAA, 2017). Most of the cultivated area used for GM crops worldwide is primarily committed to maize, soybean, cotton and oilseed rape.

At present, only one GM crop is authorised for cultivation in the EU. This is the insect resistant maize MON 810. However, several Member States of the EU have recently used Directive (EU) 2015/412 to restrict and prohibit cultivation of GMOs in their territories (EU, 2015). In contrast, many GM plants are approved in the EU for import to be used as food and feed, and to generate derived products. Most GM plants are used as animal feed. In Norway, no GM plants are approved for cultivation nor for import as food or feed. In addition, cut flowers of five carnation varieties have been approved for the EU market for ornamental use (EU, 2019b). Cut flowers of the same five carnation varieties are also approved in Norway.

GM plants with herbicide tolerance (HT) traits, frequently stacked with insect resistance traits, have constituted a large proportion of the GM crops cultivated outside the EU over the last two decades. In 2015, approximately 85% of the total area devoted to these crops was planted with GMHT crops, a large fraction being glyphosate tolerant.

Glyphosate based herbicides kill plants by blocking the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an enzyme vital to the biosynthesis of aromatic amino acids. There are two main approaches to modify plants to be glyphosate tolerant. One method is to transform plants to express a soil bacterium gene that produces a form of EPSPS with low affinity for glyphosate. Another method is to transform them with a different soil bacterium gene that encodes a glyphosate detoxifying enzyme.

Other examples of broad-spectrum herbicides used in conjunction with GMHT crops are dicamba and glufosinate-ammonium. Dicamba resembles the plant hormone auxin which accelerates growth of plants until eventually killing them. Glufosinate-ammonium kills plants by inhibiting the enzyme glutamine synthetase, vital to plant nitrogen metabolism and ammonia detoxification. To counter development of weed resistance, GMHT crops are systematically crossed to tolerate more than one broad-spectrum herbicide, e.g. dicamba and glyphosate, often along with insect resistance traits.

Cultivation of GMHT crops, together with complementary use of broad-spectrum herbicides, has significant impacts on crop management strategies and agricultural practices. GMHT crops permit the use of broad-spectrum herbicides, as an in-crop selective herbicide to control a wide range of broadleaf and grass weeds without sustaining crop injury. This weed management strategy enables post-emergence spraying of established weeds, and gives farmers more flexibility to choose spraying times, in comparison with the pre-emergence treatments of conventional crops. GMHT crops also facilitate low or no tillage cultural practices, and thereby reduce soil erosion.

The broad-spectrum herbicide(s) that are used with GMHT crops are sprayed directly on the plant canopy. Also, the spraying often takes place later in the growing season than is the case with the selective herbicides that are associated with conventional crops. Levels of individual herbicide residues and their metabolites may therefore potentially be higher in plants with tolerance to herbicides, compared to that of plants produced by conventional farming practices.

At present, the permitted maximum residue levels (MRLs) of pesticides are identical for GM and conventional crops in the EU. MRLs are defined as the upper levels of pesticide residues that are legally permissible in or on food or animal feed when pesticides are applied correctly (Good Agricultural Practice – GAP). The level of residues found in food must be safe for consumers and must be as low as possible. However, data on residue levels of herbicides and pesticides, including their metabolites, in GM foods are not included in the documentation provided by the applicants when seeking authorisation of their GM products, since this information is not requested in the applications. Instead, this type of data should in the EU be considered when producers of plant protection products apply for their use on various type of crops.

The Norwegian Scientific Committee for Food and Environment (VKM) has repeatedly reported information on residue levels of target herbicides, and metabolites, as a data gap in the risk assessments of food and feed from GMHT crops. VKM has pointed out that more research is needed to elucidate whether the genetic modifications used to make a plant tolerant against certain herbicide(s) may influence the residue levels or metabolism of the herbicide in question or of other plant protection products. Moreover, information is needed on whether changes in the spectrum of metabolites may result in altered toxicological properties.

At present, these questions fall outside the remit of VKM’s risk assessments of genetically modified plants. However, VKM considers it necessary to obtain a summary of the status of knowledge on these matters, and therefore initiated this project.

Although there are several types of GMHT crops, some tolerant to multiple herbicides, the current opinion will mainly focus on GMHT crops tolerant to glyphosate, since this is the most commonly used active ingredient for which GMHT crops have been developed.

This opinion focuses on maize, soybean and oilseed rape. Maize and soybean were chosen since they are the two major GM food and feed crops in the world. Oilseed rape was included since conventional oilseed rape is grown in Norway.

Terms of reference (ToR)

There is a need for clarification on whether or not the use of plant protection products with genetically modified herbicide tolerant (GMHT) crops represents an increased health risk to consumers compared to crops grown under conventional agricultural practices. Increased health risk can occur due to increased levels of herbicide residues and/or their metabolites in food and feed.

In addition, the genetic modifications used to make a plant tolerant against certain herbicide(s) may influence the metabolism of the intended herbicide(s), as well as the metabolism of other plant protection products. It therefore needs to be clarified in which cases, if any, such changes in the nature and/or magnitude of residues are likely to occur.

In case any novel metabolites are formed in GMHT crops, there may also be a need for clarification of their potential toxicity.

The scientific assessment should cover:

ToR 1. A comparison between common weed control practices used with glyphosate tolerant crops and those used with conventional crops

ToR 2. Residue levels of glyphosate, its metabolites and other relevant herbicides used on glyphosate tolerant and conventional crops

ToR 3. A description of how the genetic modification(s) used to make a plant tolerant against glyphosate may influence the metabolism of glyphosate or other plant protection products

ToR 4. An evaluation of whether possible changes in the spectrum of metabolites may have implications for the toxicity of glyphosate tolerant crops

Assessment

1 Introduction

This opinion presents a scientific assessment prepared by an appointed project group. The Norwegian Scientific Committee for Food and Environment (VKM) initiated this project. The Scientific Steering Committee has assessed and approved the opinion.

The purpose of the assessment is defined by the ToR described above. This is not a risk assessment of genetically modified herbicide tolerant (GMHT) crops, nor an assessment of possible health effects of glyphosate and/or its metabolites.

Environmental impact on soil and environment surrounding agricultural lands, e.g. from herbicide residues, are not included in the ToR of this opinion.

2 Data collection and literature searches

Separate literature searches were performed for each of the questions in the ToR. The project group discussed and agreed on the search terms and databases to be used together with a senior librarian at the Norwegian Institute of Public Health, who performed the searches. The literature searches are further described below. Full search strategies are included in Appendix I-IV.

2.1 A comparison between common weed control practices used with glyphosate tolerant crops and those used with conventional crops (ToR 1)

For this topic, literature searches were performed in Medline, Embase, ISI Web of Science and AGRICOLA. These databases were chosen to ensure comprehensive study retrieval. No restrictions in language or time period were used in the search. The literature searches were performed on March 9 2018.

The main searches identified 1027 articles after duplicates were removed. In the primary screening, titles and abstracts of all publications retrieved were independently screened against the inclusion criteria.

Inclusion and exclusion criteria:

- Inclusion criteria:
    - Publication type – primary research studies, review papers, systematic reviews, meta analyses and risk assessments
    - Language: English, Norwegian, Swedish, Danish or German, and other languages with English abstract
    - Papers that describe weed control and agricultural practices (herbicide use, planting, growth and harvest) in both genetically modified (GM) and conventional maize, soybean and oilseed rape

- Exclusion criteria:
    - Editorials and commentaries
    - Papers not describing the relevant agricultural practices

Articles that did not appear to meet the inclusion criteria were excluded from further analysis. In situations where it was unclear whether the publication was of relevance to the study, it was retained for further screening. Full text articles that passed the primary screening were retrieved and compared against the inclusion criteria and assessed for relevance and quality.

The primary and secondary screenings as well as quality assessment of papers were performed independently by two members of the project group. Potential disagreements were solved in the project group.

The primary screening resulted in 119 articles, of which 4 papers passed the secondary screening and were included in the opinion.

In order to strengthen the data basis of the opinion, and because the systematic literature search with the chosen databases returned fewer relevant hits than anticipated, additional manual searches for papers and relevant grey literature were also performed. Manual searches included snow-balling, i.e. articles that were referred to in papers found in the main literature, searches via Google, Google Scholar and PubMed via EndNote. The manual searches resulted in 44 relevant papers and documents included in the opinion (Figure 1.)

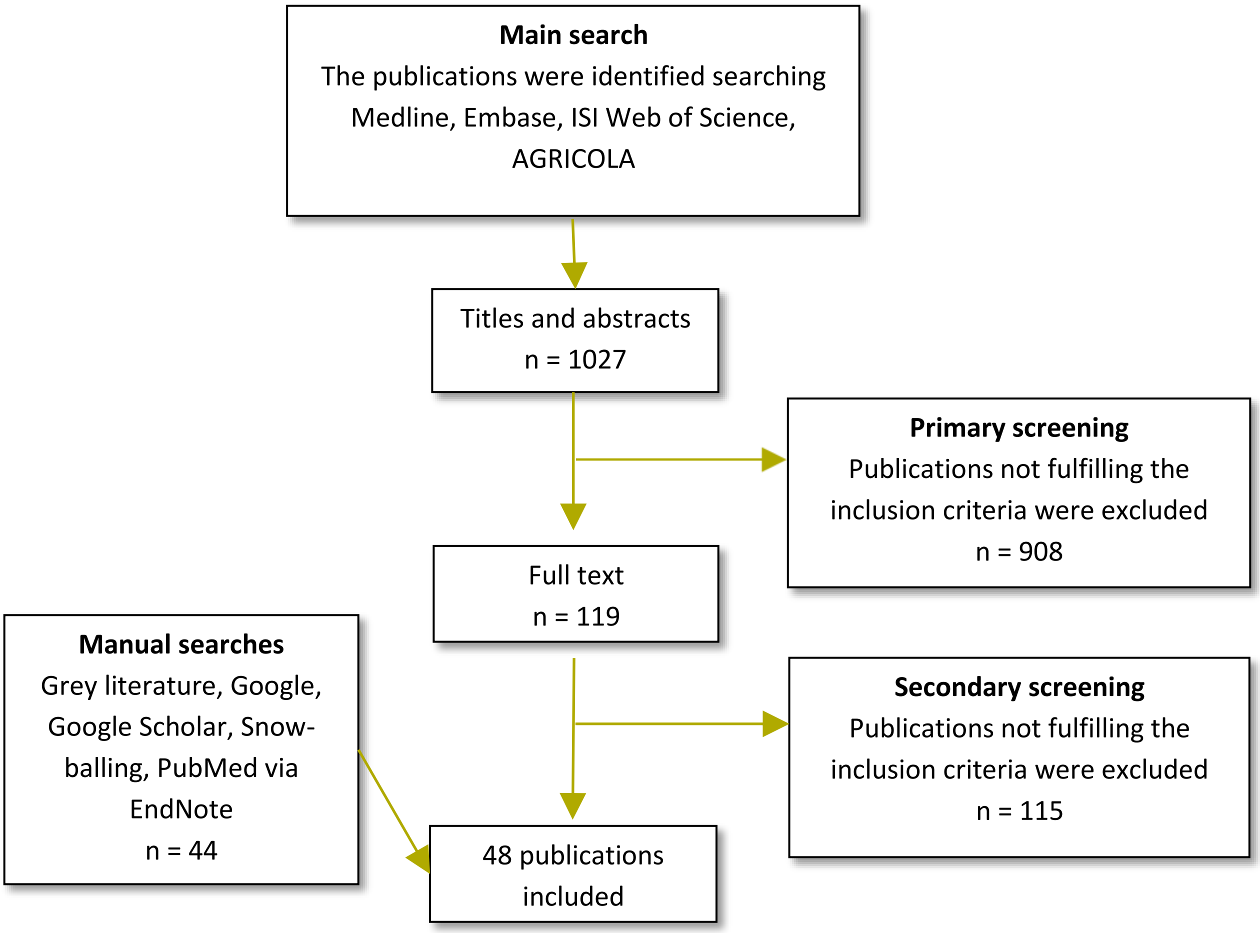


Figure 1. Flowchart for the literature search on comparison between common weed control practices used with glyphosate tolerant crops and those used with conventional crops and the subsequent selection of publications included in this part of the assessment.

2.2 Data on residue levels of glyphosate and its metabolites, as well as other relevant herbicides used on conventional crops and glyphosate tolerant crops (ToR 2)

2.2.1 Literature search for data on residue levels of glyphosate and its metabolites in glyphosate tolerant crops and conventional crops

For this topic, literature searches were performed in Medline, Embase, ISI Web of Science, Scopus, Cochrane Database of Systematic Reviews and JSTOR. These databases were chosen to ensure comprehensive study retrieval. No restrictions in language or time period were used in the search. The literature searches were performed on February 28th 2018.

The main searches identified 546 articles after duplicates were removed. In the primary screening, titles and abstracts of all publications retrieved were independently screened against the inclusion criteria.

Inclusion and exclusion criteria:

- Inclusion criteria:
    - Publication type – primary research studies, review papers, systematic reviews, meta analyses and risk assessments
    - Language: English, Norwegian, Swedish, Danish or German, and other languages with English abstract
    - Publications must include measurements/numbers on residues of glyphosate and/or its metabolites in maize, soybean or oilseed rape
- Exclusion criteria:
    - Editorials and commentaries
    - Measurements on residues in soil or water or processed products of maize soybean and oilseed rape
    - Methodological papers with spiked samples

Articles that did not appear to meet the inclusion criteria were excluded from further analysis. In situations where it was unclear whether the publication was of relevance to the study, it was retained for further screening. The primary screening was performed independently by two members of the project group. Potential disagreements were solved in the project group.

The full text of articles that passed the primary screening was retrieved for secondary screening. In this screening, the full text articles were reviewed and compared against the inclusion criteria. The secondary screening was performed by one project member.

The secondary screening resulted in 13 articles. Of these, 11 were research articles, one was a review and one was an opinion by the European Food Safety Authority (EFSA).

In addition, another EFSA opinion and five monitoring reports were added by manual search (Figure 2).

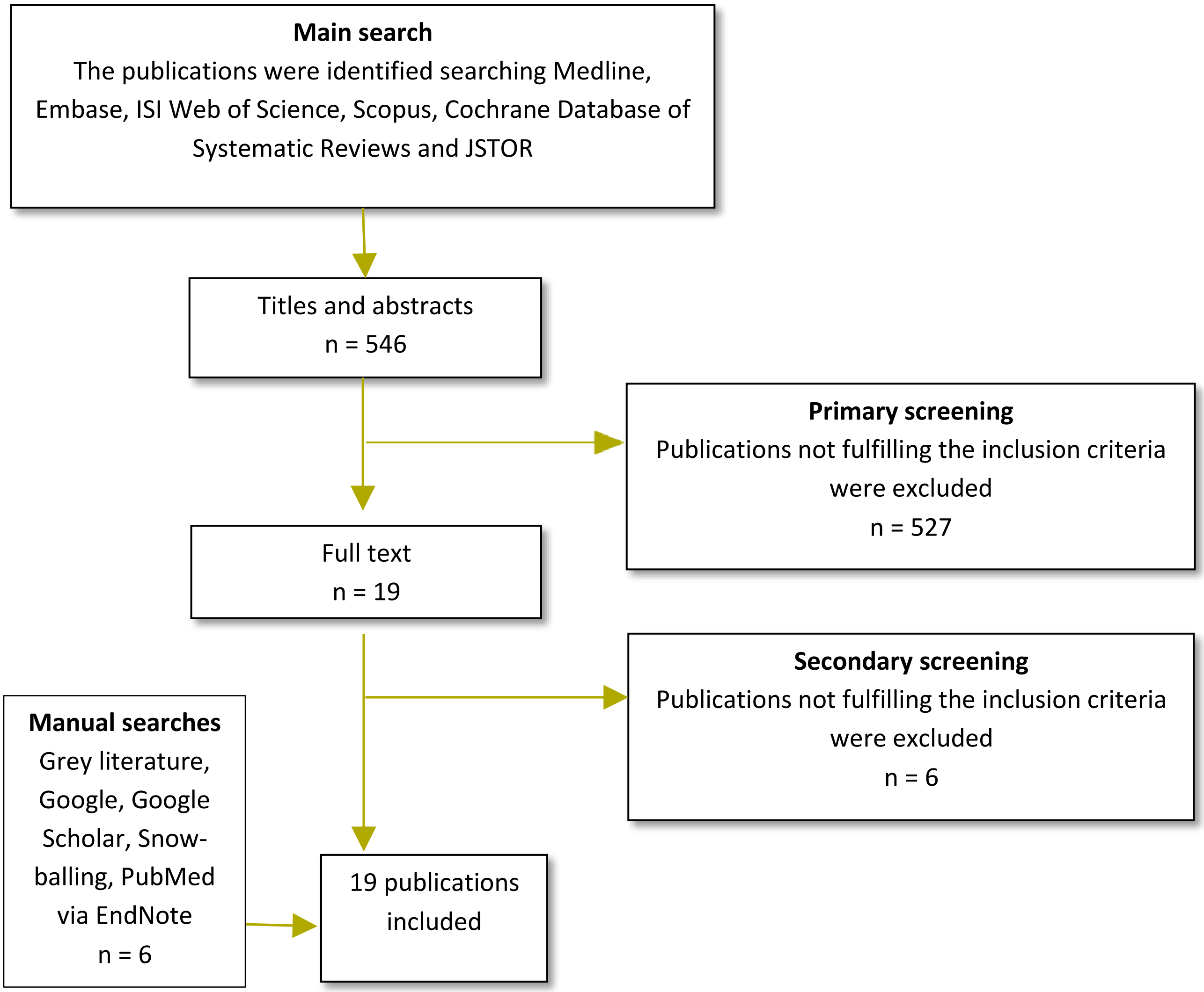


Figure 2. Flowchart for the literature search for data on residue levels of glyphosate and its metabolites in glyphosate tolerant crops and conventional crops.

2.2.2 Data on residue levels of other relevant herbicides used on conventional crops and glyphosate tolerant crops

For this topic, literature searches were performed in Medline, Embase and ISI Web of Science. No restrictions in language or time period were used in the search. The literature searches were performed on May 22nd 2018.

The main searches identified 134 articles after duplicates were removed. In the primary screening, titles and abstracts of all publications retrieved were independently screened against the inclusion criteria.

Inclusion and exclusion criteria:

- Inclusion criteria:
  - Publication type – primary research studies, review papers, systematic reviews, meta analyses and risk assessments
  - Language: English, Norwegian, Swedish, Danish or German, and other languages with English abstract
  - Publications must include measurements/numbers on residues on either of the 10 selected conventional herbicides in GM and/or conventional maize or soybean
- Exclusion criteria:
  - Editorials and commentaries
  - Measurements on residues in soil or water or processed products of maize and soybean
  - Methodological papers with spiked samples

Articles that did not appear to meet the inclusion criteria were excluded from further analysis. In situations where it was unclear whether the publication was of relevance to the study, it was retained for further screening. The primary screening was performed independently by two members of the project group. Potential disagreements were solved in the project group.

The full text of articles that passed the primary screening were retrieved for secondary screening. In this screening, the full text articles were reviewed and compared against the inclusion criteria. The secondary screening was also performed independently by two members of the project group.

The primary and secondary screening resulted in only one article (Figure 3).

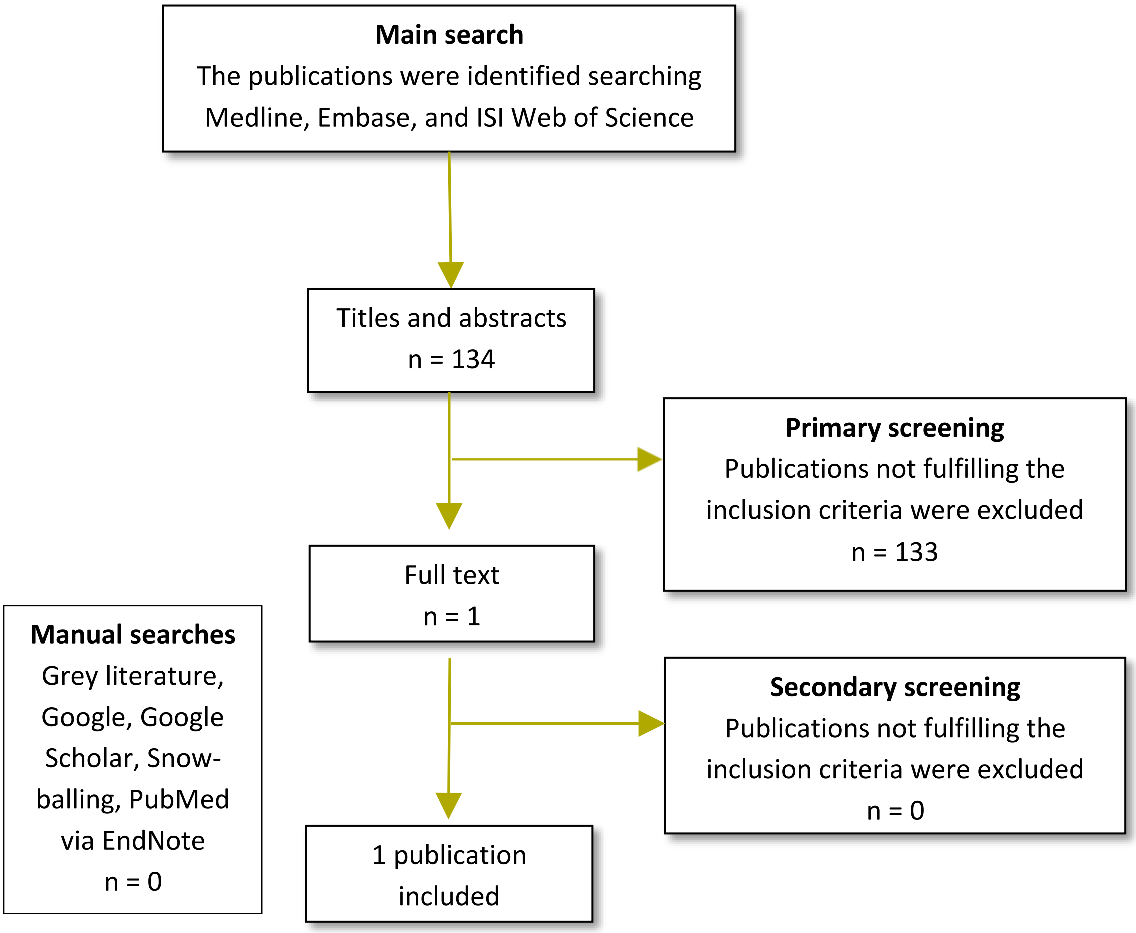


Figure 3. Flowchart for the literature search for data on residue levels of relevant herbicides used on conventional crops and glyphosate tolerant crops.

2.3 A description of how the genetic modification(s) used to make a plant tolerant against glyphosate may influence the metabolism of glyphosate or other plant protection products (ToR 3)

For this topic, literature searches were performed in Medline, Embase and ISI Web of Science. No restrictions in language or time period were used in the search. The literature searches were performed on May 25th 2018. The main searches found no articles matching the inclusion criteria.

Inclusion and exclusion criteria:

- Inclusion criteria:
    - Publication type – primary research studies, review papers, systematic reviews, meta analyses and risk assessments
    - Language: English, Norwegian, Swedish, Danish or German, and other languages with English abstract
    - Publications with description of glyphosate *N*-acetyl transferase (GAT) and/or glyphosate oxidoreductase (GOX), which mention either of the 15 selected plant protection products (PPPs)
- Exclusion criteria:
    - Editorials and commentaries

The selected search strategy returned no relevant articles of enzymes when combined with the 15 PPPs. However, there were hits in each of the three databases for the enzyme GAT and/or GOX: 19 articles were found in both Medline and Embase and 54 in ISI Web of Science (92 in total). The article abstracts were scrutinised and relevant full texts were reviewed independently by two project members. Potential disagreements were solved in the project group

Three articles published by the developers of the GAT enzyme, Pioneer Hi-Bred International, were found relevant to the topic(s) of ToR 3 (Figure 4), describing the enzymatic acetylation and subsequent detoxification of glyphosate and certain chemical group affinities of the enzyme (Castle et al., 2004; Siehl et al., 2005; Siehl et al., 2007).

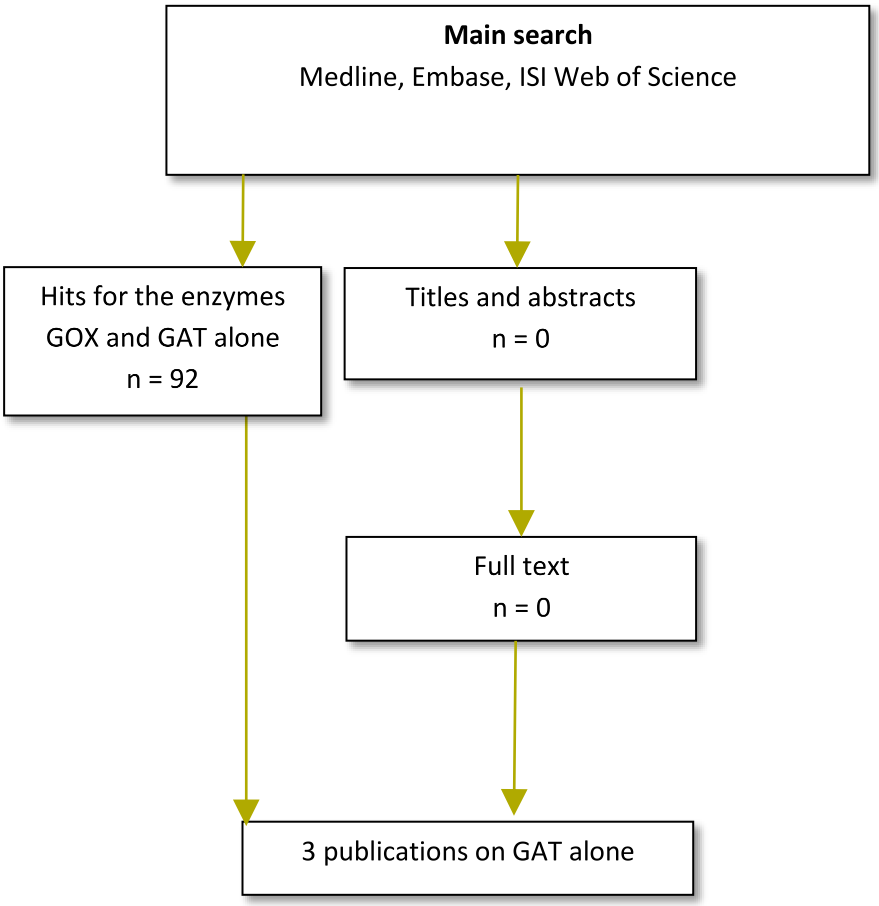


Figure 4. Flowchart for the literature search for data on enzymatic interaction of glyphosate *N*-acetyl transferase (GAT) and/or glyphosate oxidoreductase (GOX) with 15 major pesticides used on glyphosate tolerant crops. The search returned no hits when the enzymes were combined with either of the 15 pesticides, but three publications relevant to the topic(s) of ToR 3 describing the GAT enzyme and some relevant chemical group affinities, were included.

2.4 An evaluation of whether possible changes in the spectrum of metabolites may have implications for the toxicity of glyphosate tolerant crops (ToR 4)

Since the literature search on ToR 3 did not return any relevant publications, a specific literature search with additional terms for ‘toxicity’ would not have given any relevant publications either. Therefore, this search was not performed.

3 A comparison between common weed control practices used with glyphosate tolerant crops and those used with conventional crops (answer to ToR 1)

3.1 Agricultural practices in maize, soybean and oilseed rape production

Maize, soybean and oilseed rape are the main genetically modified herbicide tolerant (GMHT) edible crops in terms of global production volumes. In this chapter, the agricultural practices involved in the cultivation of conventional and GMHT crops are described, with focus on weed management strategies. Little information is available on the cultivation of such crops in Norway and other European countries. Description of agricultural practices is mainly based on information from USA, South America and Australia. However, there are large regional variations in climate affecting growing seasons and agricultural practices.

3.1.1 Maize

Maize (*Zea mays*), also known as corn in USA, is the most important cereal crop worldwide (FAOSTAT, 2018). It has a better adaptability to varying agro-ecological regions than other cultivated crops. Besides serving as human food and animal feed (mainly to cows, chickens and pigs), maize products are used in a variety of industrial applications. For example, maize starch is used in paper production. Maize oil is used in margarine, maize syrup sweeteners is used in marmalade and maize syrup solids is used in instant non-dairy coffee creamer (Wilkes, 2004).

Yield losses in maize are to a large extent caused by competition with weeds. Weed interference is a severe problem, especially in the early part of the growing season, due to slow early growth rate and wide row spacing (Kremer, 2004). A significant area of maize production is grown for whole plant silage, especially for dairy farmers and feedlots. In USA, 40% of maize goes to ethanol production.

Brazil is one of the largest producers and exporters of maize. There, the crop is grown during two seasons. The first crop is harvested in spring/summer and the second is harvested in the summer/fall (late harvest). Most of the late harvest area is sown in dryland and in succession to a summer culture, often soybeans (Marca et al., 2015).

Maize production in Europe differs depending on the geographical area. In Norway, there is only a marginal cultivation of maize, ~280 ha (Bioforsk, 2013). In Denmark, Netherlands and north of France most of the maize is harvested for silage, i.e. whole ensiled maize plants. Mainly grain maize is harvested in Southern and Eastern Europe, and used as food and feed (Figure 5).

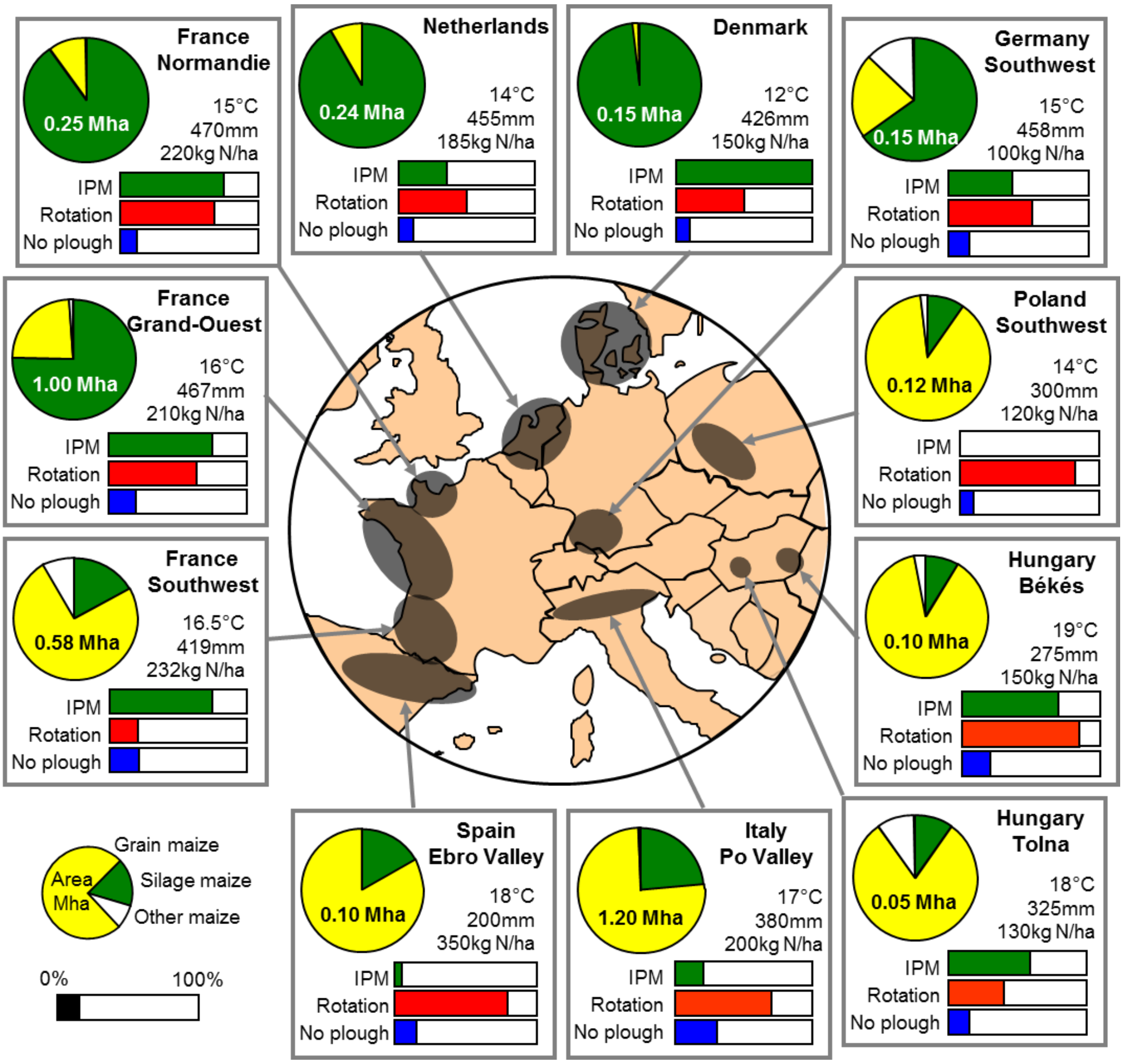


Figure 5. Maize production characteristics in 11 regions in Europe. **Pie diagrams:** Maize production type: Silage (green), grain (yellow) and other (white); Numbers in diagrams: total maize area in the region (in million hectares); Numbers outside diagrams: Average temperature (°C) and precipitation (mm) from April to October and fertilizers (synthetic and organic) applied per year (kg nitrogen input per ha); **Bar diagrams:** Percentage of maize area under integrated pest management (IPM) including organic, crop rotation (no maize after maize), low tillage (including no tillage) soil management versus ploughing. Full bars represent 100% (adopted with the authors' permission from (Meissle et al., 2010).

Integrated pest management (IPM) is implemented as a general practice by EU legislation (EU, 2009). Weed control is the most important crop protection for maize cultivated in Western Europe since integrated weed management is an important component of integrated pest management.

Planting

Maize can be direct drilled or sown into a cultivated soil bed (Figure 6). In some countries, maize is still broadcasted. The desirable distance between rows is 65 - 75 cm, with a minimum of 15 cm between plants in the same row. Maize does not germinate at soil temperatures below 10°C and ideally, planting should occur once the soil temperature has reached 12°C. Usually, planting depth of maize varies from 5 to 10 cm, depending on the soil type and planting date. Planting should be shallower in heavier soils than in sandy soils. Plant population per ha varies considerably around the world, depending on crop varieties, rainfall, soil fertility and other environmental variables. In very dry environments (below 500 mm rainfall/year) plant densities ranging from as low as 15000 to 25000 plants/ha can be found. However, at more favorable environmental conditions or in irrigated areas, population densities between 50000 to 100000 plants/ha give the optimum grain production (Davis et al., 1987).

Figure 6. Direct-drilled maize field (Colourbox).

Growth

The length of the growing season is important for growth of crop varieties, especially in cooler production areas. The cooler environment creates large differences among crops in the period available from planting to flowering and physiological maturity.

Successful cultivation of maize depends largely on the efficacy of weed control. Weed control during the first six to eight weeks after planting is important, because weeds compete with the crop for nutrients and water during this period. Annual yield losses occur as a result of weed infestations in cultivated crops. Examples of uncontrolled weeds have caused field maize yield reductions in research trials that range from 24% to 56% (38% average). Research has shown that maize can tolerate a certain level of weed pressure and that control strategies should only be implemented when the potential yield losses caused by the weeds exceeds the cost of control (i.e. economic threshold concept) (Garcia, 2015).

Harvest

A crop is ready to be harvested for fodder or silage use, theoretically, about 10–14 days before physiological maturity—this will be about 3–4.5 months after planting. If growing maize for silage, a forage harvester and wagon will be required (Figure 7), and appropriate equipment if the silage is to be baled. Fodder and silage may be kept and used at the farm, generally in large pits, or it may be wrapped in plastic and stored as bales.

Figure 7. Harvesting maize for silage (Colourbox).

A crop cultivated for grain production will be ready about 4.5–6.0 months after planting. Maize grain is harvested with a combine harvester that has a specialised corn front. Maize for grain must be handled carefully during harvest to minimise cracking and breaking of the grain. This can be managed through correct settings and operation of the harvester; such as, using belt conveyers instead of augers to move grain, keeping augers full to minimise grain movement; and avoiding dropping the grain from heights to avoid impact damage (Figure 8) (Moore et al., 2014).

Figure 8. Maize grain harvest (IStock).

3.1.1.1 Conventionally grown maize

After atrazine was banned for plant protection within the EU, weed management relies on the use of a combination of herbicides applied before and after planting of the crop. A multitude of herbicides are labeled for use in maize fields and can be applied pre-plant, incorporated, pre-emergence and post-emergence. One example of chemical treatments as a conventional strategy in Western Europe were 1. Flufenacet + terbuthylazin + sulcotrion + foramsulfuron + isoxadifen-ethyl, 2. Bentazon + terbuthylazin + dimethenamide-P + mesotrione. 3. S-metholachloor + terbuthylazin + mesotrione + nicosulfuron and 4. Pethoxamide + mesotrione + terbuthuylazin + nicosulfuron (Latre et al., 2015). In the European project PURE (http://www.pure-ipm.eu), conventional weed management on-farm in maize was compared with integrated weed management in three countries (Germany, Slovenia and Italy). This included pre-emergence and post-emergence use of herbicides. The use of pre-emergence herbicides in Italy included mesotrione, S-metolachlor and terbutylazin, while post-emergence herbicides differed more with different combinations of topramezone, dicamba, dimethenamid-P, foramsulfuron/isoxadifen-ethyl, nicosulfuron, mesotrione, and prosulfuron (Vasileiadis et al., 2015). The overall conventional weed management provided a significant higher weed control than integrated weed management, but all integrated weed management had a lower pesticide load in terms of herbicide use. None of the strategies included use of glyphosate.

In USA, to effectively control emerged common ragweed with herbicides before maize emergence, atrazine combined with one of the following herbicides - 2,4-D ester, dicamba, glyphosate, or paraquat - is recommended. Where glyphosate resistance is suspected, glyphosate is combined with either 2,4-D ester or dicamba and increased paraquat rates with increases in weed size.

Pre-emergence herbicides recommended in Australia are atrazine in combination with methalochlor, used to control summer annual grasses and broadleaf weeds. If broadleaf weeds grow later in the season, post-emergent options are available with, e.g. dicamba and 2,4-D. Primsulfuron can be used post emergent to control couch grass (Garcia, 2015).

3.1.1.2 Glyphosate tolerant maize

Recent concerns about herbicide resistant weeds have caused many farmers to reconsider a zero-tolerance policy for weeds. Herbicide use in maize differs substantially from that in soybean, both in the types of herbicides used and the variety of herbicide sites of action. The maize yield is particularly negatively influenced by early season weed competition. Maize is planted in wide rows, and the resulting penetration of light allows weed germination over a long period of time. For these reasons, post-emergence application of glyphosate is not as beneficial in maize as it is in soybean. Farmers manage weeds with pre-plant or pre-emergence herbicides to obtain the best maize yields. Historically, they have used atrazine and chloroacetamide herbicides to control emerging weeds after planting the maize. Even after glyphosate tolerant maize was widely adopted, most maize growers have continued to use herbicides, such as atrazine and chloroacetamides, followed by application of post emergent herbicides, such as glyphosate, to provide good weed control and maximise yield potential (USDA-APHIS, 2014).

Due to lack in both diversity of weed management and type of herbicide used, weeds have developed herbicide resistance (Owen et al., 2010). Approximately 30% of the maize planted in USA is cultivated in a no-tillage system, and herbicides are the primary strategy to manage weeds (USDA-ERS, 2010). In some states in USA, approximately 80% of the maize is traditionally planted no-tillage, while in some states, such as Arkansas, maize is planted on beds that are prepared in the fall. Therefore, spring tillage as a weed management strategy has been minimal (Owen et al., 2015). In Midwestern USA, typically some tillage (e.g. chisel plow) is done after harvest in the fall and generally one tillage trip (i.e., disc harrow) is performed in the spring immediately prior to planting (Owen et al., 2015). The tillage done in Midwestern USA improves overall weed management by burying weed seeds, which helps to control glyphosate resistant *Amaranthus palmeri*. If tillage is not performed prior to planting, a non-selective herbicide is applied, often in combination with tank mixed atrazine. In most cases, a second post-emergence application is applied which contains glyphosate and atrazine. Another integrated weed management option that has recently been adopted by growers in Southern USA to manage glyphosate resistant *A. palmeri* is post-maize-harvest weed control (Owen et al., 2015).

A. palmeri often emerges as the maize crop dries in early July and the weed can produce huge amounts of seed before a killing frost. Therefore, farmers have applied paraquat plus residual herbicides, like atrazine, to kill the late germinating *A. palmeri* or are mowing and tilling small *A. palmeri* after maize harvest. In summary, weed control in maize fields in USA is heavily reliant on herbicides. There is minimal row cultivation, and adoption of other alternative tactics (e.g., cover crops) is limited at best. In recent years, more tillage has been integrated into maize production systems to supplement weed management, largely on account of herbicide resistant weeds (Colbach et al., 2017a; Colbach et al., 2017b).

With GM crops of maize in rotation with GM soybeans, volunteer maize will germinate to quite high levels in the following crop (Marca et al., 2015). The volunteer maize plants may damage the subsequent crop and/or result in significant losses (Albrecht et al., 2013). This requires chemical control measures with other herbicides than glyphosate. The herbicides paraquat, haloxyfop-p-methyl, tepraloxydim, cyhalofop-butyl, fluazifop-p-butyl, sethoxydim, fenoxaprop-pethyl and imazethapyr are efficient in controlling maize plants resistant to glyphosate (Marca et al., 2015).

In addition to herbicides, other pesticides can be used in maize production. Approximately 12% of the maize-planted areas were treated with insecticides in 2010, with the most abundantly applied being tefluthrin for control of corn rootworm (3%), cyfluthrin for corn rootworm, earworms and European corn borer (2%), lambda-cyhalothrin for European corn borer (2%), bifenthrin for grubs, wireworms, seed-corn maggot, and cutworms (2%) and tebupirimphos for corn rootworm and seed corn maggot (2%) (USDA-APHIS, 2014).

3.1.2 Soybean

Soybean (*Glycine max*) is one of the most important food crops globally (FAOSTAT, 2018). The soybean originates from Asia. The top producers today are USA, Brazil and Argentina. Commercially important products commonly made from soybeans include protein powders, textured vegetable protein, soybean vegetable oil and livestock feed. Soybean meal is used in food and animal feed, whereas the oil fraction is used mostly in food, but also for non-food, e.g. industrial purposes and biodiesel (NCSPA, 2018).

Planting

Figure 9. Tractor with multirow planter (Colourbox).

Like many field crops soybeans are grown from seeds planted in rows. Soybean seeds can be planted in spring two to three weeks after the average last frost date when the soil has reached at least 15° C. In North America (North Carolina), farmers plant soybeans from the beginning of May to July. Seeds are planted in cultivated or tilled land by using a tractor and a planter, which deposits the seeds about 3-4 cm deep in rows that are up to 45 cm apart (NCSPA, 2018).

When a farmer uses the "no-till" method, the land is not cultivated and the seeds are planted directly into the stubble left over from the previous crop. The "no-till" method conserves moisture and greatly decreases the possibility of soil erosion. Sometimes tillage is required, especially for dealing with fields infested with weeds.

Large tractors and multi-row planters are used to plant many rows at the same time (NCSPA, 2018) (Figure 9).

Growth

About seven days after planting, the soybeans sprout and small plants begin to grow. Weeds that grow faster than the soybean plants constitute a major threat to the young plants. The weeds will shade seedlings from light and reduce the level of nutrients available to soybean plants and cause reduced yields.

Weeds will also produce seeds and be harvested with the soybeans and thereby reduce value of the harvest. The soybean plants bloom in July till August and are self-pollinating.

Harvest

Figure 10. When the combine tank is full, the soybeans are emptied into a grain wagon (IStock).

The soybeans start to mature in late September. In mid-October and November, the leaves turn brown and fall off. The soybeans are harvested with a combine, which cuts and collects the soybean plants. The combine separates the soybeans from their pods and stems and collects the soybeans into holding tanks which are emptied into a grain truck (Figure 10). The soybeans are transported to a processing facility, where the soybean meal (dry matter) and oil fraction are separated.

3.1.2.1 Conventionally grown soybeans

Although GM soybeans dominate the soybean cultivated areas in USA, there are areas in South Dakota where conventional soybeans are grown (Shaffer, 2016). The approach to weed management in conventional soybeans requires a higher level of management skills compared with those used for GM soybeans. In order to ensure success in conventional soybeans, farmers are recommended to avoid fields with a high population of weeds (Shaffer, 2016). Fields with a history of poor control associated with resistance to acetolactate synthase (ALS) inhibitors or protoporphyrinogen oxidase (PPO) inhibitors should also be avoided (Martin and Green, 2018). Soybean injury can occur with certain herbicides, particularly when stressed from adverse environmental conditions. Also, certain pesticide additives can enhance injury from post-emergence herbicides. Some soybean herbicides can be persistent in soil and potentially injure rotational crops. This can occur with herbicides containing chlorimuron, imazaquin, imazethapyr and clomazone (Martin and Green, 2018). The main options for pesticide application are burndown, pre-emergence, post-emergence and fall applications (Shaffer, 2016).

- Burndown herbicide options (controlling emerged weeds at planting)

To achieve a clean field for the soybean seeds, a burndown herbicide that controls a broad spectrum of weeds, such as glyphosate, paraquat or glufosinate, before or at planting is recommended. Other herbicides that may add to weed control could include 2,4-D ester and a metribuzin product. Burndown herbicides are especially recommended for no-till planting.

- Pre-emergence herbicide options

Starting with a clean field, farmers are advised to control early weeds to give the soybeans a head start. Dependent on weed pressure and species the following pesticides are recommended: Sulfentrazone, imazethapyr, sulfentrazone, cloransulam, chlorimuron, metribuzin, flumioxazin chlorimuron, flumioxazin, cloransulam, flumetsulam, imazaquin and flumioxazin.

- Post-emergence herbicide options

After crop emerging, the weeds can be controlled when they are 5 – 10 cm. Post-emergence herbicide options include fomesafen, fomesafen + clethodim and fenoxaprop (all being grass herbicides). Other options could include chloransulam-methyl chlorimuron or chlorimuron and thifensulfuron-methyl.

Applications of herbicides in the fall are not as effective as burndown in spring and not recommended. There are options for fall application with glyphosate and 2,4-D or 2,4-D and a low rate of a chlorimuron-containing product.

3.1.2.2 Glyphosate tolerant soybeans

The introduction of herbicide tolerant crops into an existing cropping system was accompanied by changes in chemical weed control techniques. This could be done either directly or indirectly by changes in other agricultural practices at the field level (Beckert and Dessaux, 2016).

The only GM glyphosate tolerant crop grown commercially in Europe was soybean 40-3-2, which was grown in Romania until 2007 (Kleter et al., 2008), when Romania joined the EU.

Soybeans with tolerance to glyphosate are planted on more than 95% of the land in USA where soybeans are cultivated, and glyphosate is used on 98% of that area (Owen et al., 2015). No-tillage soybean production approaches 50% or more of the area planted with soybeans and is a higher percentage than for all other row crops planted in USA. The trend to adopt no-tillage soybean production has increased during the last decade and this trend is likely partially attributable to the adoption of GM soybeans.

Also in Argentina, the widespread adoption of glyphosate tolerant soybeans is linked to the incorporation of no-tillage technology. In 2007, around 75% of the first-crop soybean cultivated area and 83% of the second-crop soybean cultivated area were managed with this technology. No-tillage has also greatly facilitated the planting of soybeans immediately following the wheat harvest, allowing two crops in the same year (Lence, 2010).

Soybean weed control in USA, though still relying heavily on glyphosate, has begun to integrate more alternative herbicides than 10-15 years ago. Reliance almost exclusively on one herbicide is changing slowly to combinations of herbicides that are effective on herbicide resistant weeds. A major cause for the increased use of herbicides in soybean fields is the rapid evolvement of glyphosate resistant weeds in regions cultivating GM glyphosate tolerant crops (Meyer and Cederberg, 2010). Changes in soybean weed management are more apparent in soybean production in Southern USA than in the Midwestern. In the Midwestern soybean production, herbicide use still reflects the dominance of glyphosate. Two pass programs consisting of pre-emergence residual herbicides followed by post-emergence herbicides have shown better effects than single post-emergence treatments (Peterson et al., 2017).

The use of insecticides in soybeans is not likely to be significantly different between GMHT and conventional crops. A 2006 survey performed by USDA-NASS, found that insecticides were applied to 16% of the 72.9 million soybean acres planted in surveyed states in 2006. Of the 12 reported insecticides, the three most common - lambda-cyhalothrin, chlorpyrifos, and esfenvalerate - were applied to 6%, 5% and 3% of the planted acres, respectively (USDA-APHIS, 2014).

3.1.3 Oilseed rape

Oilseed rape (*Brassica napus oleifera*) constitutes approximately 450 million annual tons, or 20%, of the global grain production (Carré and Pouzet, 2014). In Norway, less than two percent (~3220 ha in 2018) of cultivated land is planted with oilseed rape (SSB, 2019).

Canola is a special type of oilseed rape. Canola, originally a trademark name of the Rapeseed Association of Canada, is a word derived from "Can" in Canada and "ola" from other vegetable oils low in non-edible acids. Less than 2% of the total fatty acids in canola varieties must be erucic acid. In Europe, oilseed rape varieties with a higher percentage of erucic acid are grown for industrial purposes; their oils being used as lubricants. Canola consists of three species: *Brassica napus*, known as Argentine canola, *Brassica rapa* subsp. *oleifera*, known as turnip rape (or Polish Canola) and *Brassica juncea*, known as brown mustard (quality Canola). All species belong to the *Brassicaceae* (*Cruciferae*) family, also known as the mustard family. Spring and winter annual types are available in *B. napus* and *B. rapa*. Canola varieties with tolerance to specific herbicides have been developed. Available herbicide tolerant canola include genetically modified glyphosate-tolerant and glufosinate-tolerant varieties; a conventional triazine-tolerant canola developed by traditional breeding techniques in the early 1980s, and conventionally bred "Clearfield" varieties tolerant to different imidazolinone herbicides (NDSU, 2015).

Planting

An oilseed rape seed is very small (weighing only 4 - 6 mg) and planting depth is shallower than for most grain crops. Canola needs the top 7.5 cm of soil to be moist and is planted no deeper than three cm in rows 15 cm to 45 cm apart (Farmer's-Weekly, 2014). Germination takes four to ten days. In Europe, oilseed rape varieties are grown in rotations with cereals.

Growth

The plant quickly establishes a rosette of leaves. A cluster of flower buds becomes visible in the center of the rosette and the time of seedlings to first flower range from 40 to 60 days. Flowering lasts 14 to 21 days and by the time flowering is finished, the leaves have yellowed and fallen from the plant. Seed fill is completed 35 to 45 days after flower initiation.

Harvest

Figure 91. Swathed Canola (Colourbox).

When canola plants have reached a stage when they consist of stems, stem branches and pods, the crop can be harvested. Harvesting could be done by swathing and combing, or by straight combining (Figure 11). Some farmers practice natural frost desiccation for harvesting by straight combining, or use a pre-harvest herbicide, such as glyphosate or diquat.

3.1.3.1 Conventionally grown oilseed rape

Weed control in conventionally grown canola is managed by pre-plant, pre-emergence, post-emergence, pre-harvest and post-harvest herbicide treatment. According to Canola Council of Canada’s canola grower’s manual a combination of one pre-seed weed control and one in-crop application of herbicide(s) is recommended (https://www.canolacouncil.org/). The pre-seed herbicide is often a tank mix of glyphosate as the regular herbicide together with bromoxynil or carfentrazone. Specific graminicides belonging to the acetyl-CoA carboxylase (ACCase) inhibitors may be used post-emergence. The graminicides are effective in post-emergence on a range of dicotyledons and thus represents a weed-control equivalent to the use of an acetolactate synthase (ALS) inhibitor-tolerant oilseed rape.

Cereal/GMHT oilseed rape rotations are of concern, since numerous weeds are common in both oilseed rape and winter cereals. In oilseed rape, numerous weed species are at risk for developing resistance: four of these being brassicas, three geraniums, five umbellifers (including bullwort), as well as common poppy and a number of cool-season grasses (including black-grass and the ryegrasses). Herbicides with another mode of action than inhibiting ALS are less effective or even ineffective on these species. Cross-resistance seems particularly likely to develop in grasses, brassicas and common poppy.

In Australia, most paddocks being prepared for canola will normally receive a knock-down spray of glyphosate at pre-seeding, regardless of the variety of canola grown (Oliver et al., 2016).

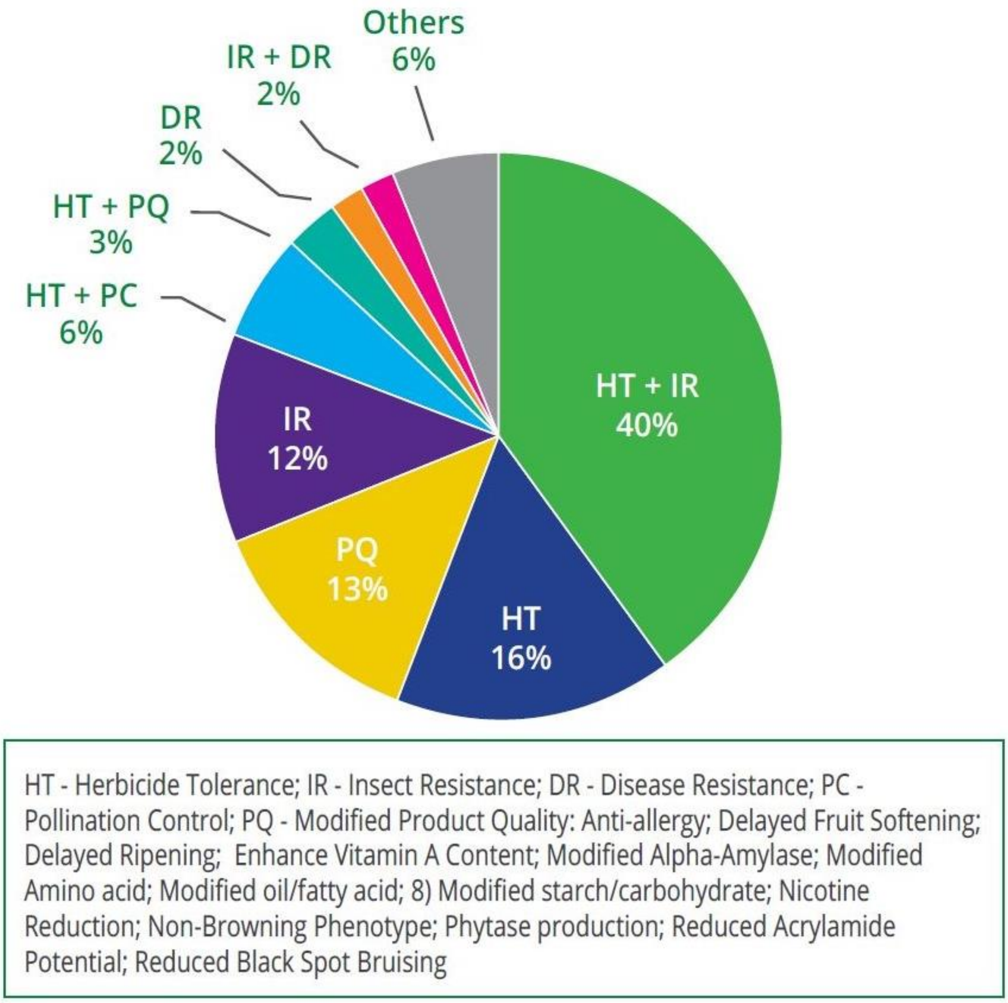
3.1.3.2 Glyphosate tolerant oilseed rape

Weeds are managed similarly in genetically modified glyphosate tolerant canola as in conventional canola; that is, with pre-seed treatment to knock down the weed before planting, pre-harvest as a desiccation treatment to make harvesting and combining easier, and post-harvest to burn off weeds after harvesting the crop. All these treatments can be done with glyphosate alone or glyphosate in combination with another herbicide. The pre-harvest treatment increases the risk of herbicide residues in the seeds. In addition to these applications, which are available for all types of canola, GM canola can also receive post-emergence application(s) of glyphosate. In an aim to reduce the risk of weed resistance development, farmers are focusing on a more integrated approach and switch annually between glyphosate- and glufosinate-tolerant crops and use additional herbicides (Benbrook, 2016).

3.2 Development of herbicide tolerant GM crops and its effects on the use of herbicides

3.2.1 Traits in genetically modified crops

To date, the dominating trait in GM crops has been herbicide tolerance (HT), followed by insect resistance (IR). GM varieties with improved product quality (e.g. modified fatty acid or amino acid composition) have been developed more recently. Often, HT and IR traits are stacked producing crops with combined resistance to herbicide(s) and insects. GM crops with HT-traits, alone or in combination with other traits, comprised 59% of the GM events approved up to 2017 (Figure 12). For the edible soybean, maize and oilseed rape, HT-traits are even more dominating, since non-stacked IR traits are most common in cotton.



HT - Herbicide Tolerance; IR - Insect Resistance; DR - Disease Resistance; PC - Pollination Control; PQ - Modified Product Quality: Anti-allergy; Delayed Fruit Softening; Delayed Ripening; Enhance Vitamin A Content; Modified Alpha-Amylase; Modified Amino acid; Modified oil/fatty acid; 8) Modified starch/carbohydrate; Nicotine Reduction; Non-Browning Phenotype; Phytase production; Reduced Acrylamide Potential; Reduced Black Spot Bruising

Figure 102. Trait distribution in approved GM events (ISAAA, 2017).

The first GMHT crops reached the market in 1996, when GMHT soybean was introduced in USA. It was followed by GMHT maize and cotton in 1997, oilseed rape in 1999 and sugar beet in 2007 (Brookes, 2014). Adoption of the GM technology has been rapid and the global area on which GM crops are grown reached close to 190 million hectares in 2017. In 2016, 88% of the GM hectarage was in the American continents, 9% in Asia, 2% in Africa and less than 1% in Europe (ISAAA, 2017) (Figure 13).

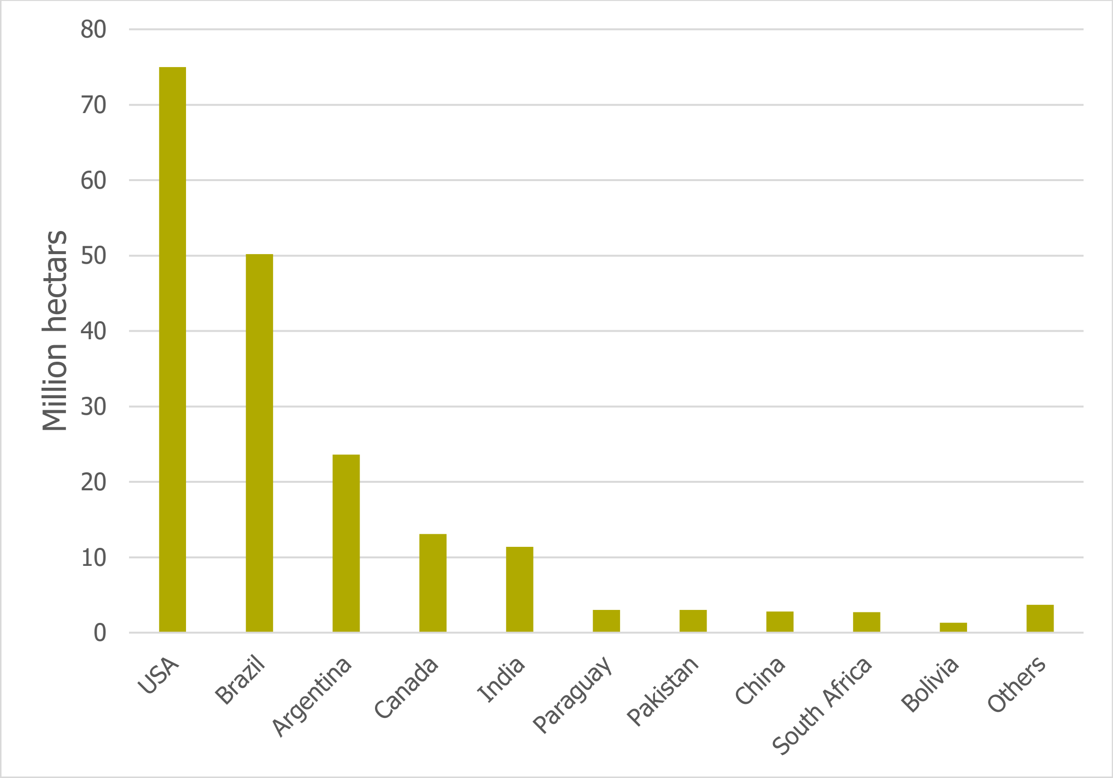


Figure 13. Global area of GM crops in 2016. Based on data from ISAAA, 2017.

The most important GM crops are soybean, maize, cotton and oilseed rape (Figure 14). In 2015, crop varieties produced by GM technology accounted for 48% of the global plantings of these crops (Brookes and Barfoot, 2017).

GM varieties of these crops have almost entirely replaced corresponding conventional crops in many countries. In USA, 93% of maize, 94% of soybeans and 100% of oilseed rape were GM in 2017. In Brazil, the adoption rate of GMHT was 97% for soybean and ~90% for maize. In Argentina, essentially 100% of soybean and 97% of maize were GMHT. Canada is a major producer of oilseed rape (Canola). Here, the adoption of GMHT reached 95% of the acres harvested in 2017 (ISAAA, 2017).

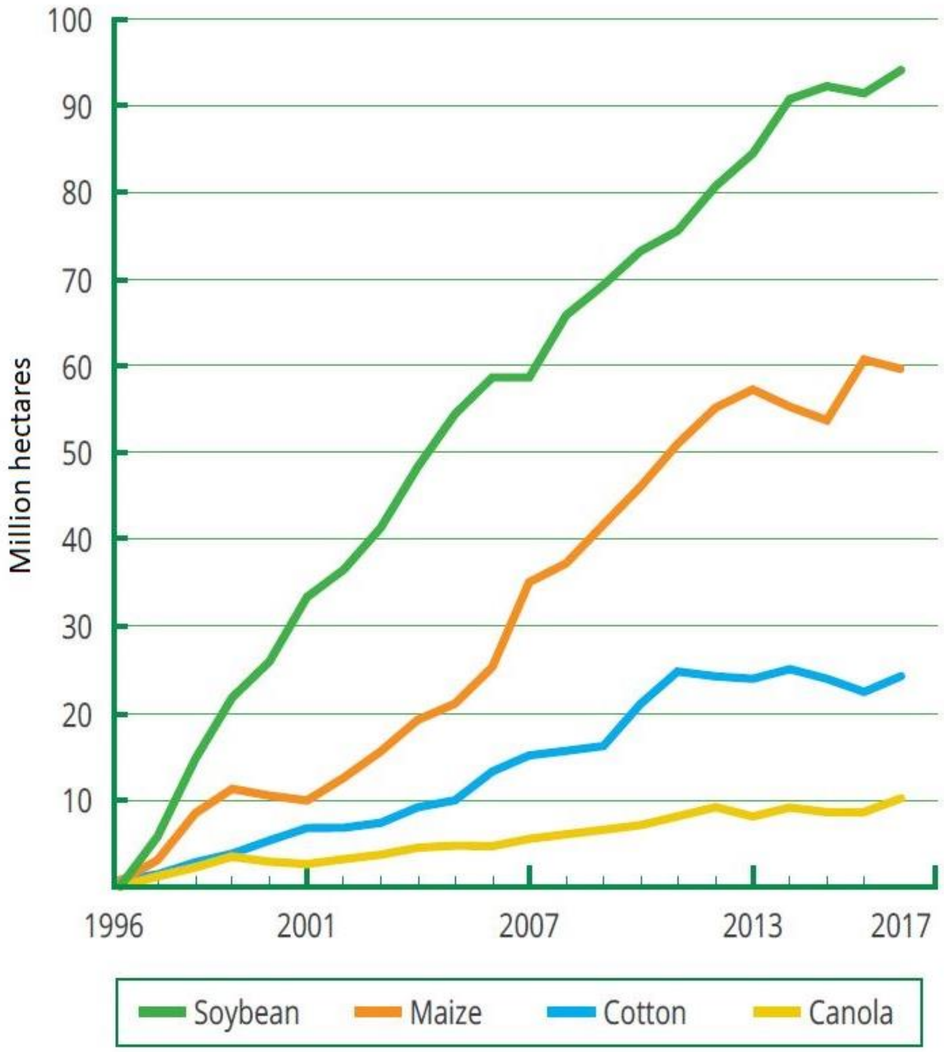


Figure 114. Global Area of Biotech Crops, 1996 to 2016: by Crop in Million hectares (ISAAA, 2017).

3.2.2 Herbicide tolerant GM crops

The most frequent trait in GMHT crops is tolerance to glyphosate. Glyphosate inhibits the enzyme enolpyruvylshikimate-3-phosphate synthase (EPSPS) in the plant chloroplast-localised pathway that leads to the biosynthesis of aromatic amino acids. There are two basic strategies that have been successful in introducing glyphosate tolerance into crop species: *i)* expression of an EPSPS enzyme with low affinity for glyphosate, and *ii)* detoxification of the glyphosate molecule (Pollegioni et al., 2011).

The second most applied HT-trait is glufosinate (phosphinothricin) tolerance which involves a gene (*pat* or *bar*) encoding an enzyme that degrades glufosinate by acetylation. This was approved for maize in USA in 1995.

With the increased awareness that extensive and repeated use of one single herbicide is likely to select for weed populations resistant to the same herbicide, the need for more proactive and diversified weed management programs has been recognised. This has stimulated the development of additional GMHT-traits, e.g. tolerance to sulfonylureas, 2,4-D, isoxaflutole, mesotrione and dicamba. These traits are often used stacked with tolerance for glyphosate. Examples of genes utilised in GMHT crops and their functions in generating tolerance are presented in Table 1.

It should be noted that there are also conventionally bred varieties available that tolerate high levels of certain herbicides (Tan and Bowe, 2011). These non-GM crop varieties are, however, not dealt with in this opinion.

Table 1. Examples of genes utilised in transgenic herbicide tolerant crops (adopted from ISAAA, March 2018)

Trait	Gene introduced	Gene source	Enzyme	Function
Glyphosate tolerance	*cp4 epsps* (*aroA:CP4*)	*Agrobacterium tumefaciens* strain CP4	Herbicide tolerant form of 5-enolpyruvulshikimate-3-phosphate synthase (EPSPS)	Decreases binding affinity for glyphosate, thereby conferring increased tolerance to glyphosate herbicide
Glyphosate tolerance	*Gat4621*	*Bacillus licheniformis*	Glyphosate *N*-acetyltransferase	Catalyzes the inactivation of glyphosate, conferring tolerance to glyphosate herbicides
Glyphosate tolerance	*Goxv247*	*Ochrobactrum anthropi* strain LBAA	Glyphosate oxidase	Confers tolerance to glyphosate herbicides by degrading glyphosate into aminomethyl-phosphonic acid (AMPA) and glyoxylate
Glufosinate tolerance	*Bar*	*Streptomyces hygroscopicus*	Phosphinothricin *N*-acetyltransferase (PAT)	Eliminates herbicidal activity of glufosinate by acetylation
Glufosinate tolerance	*Pat*	*Streptomyces viridochromogenes*	Phosphinothricin *N*-acetyltransferase (PAT)	Eliminates herbicidal activity of glufosinate by acetylation
2,4-D tolerance	*aad-12*	*Delftia acidovorans*	Aryloxyalkanoate di-oxygenase 12 (AAD-12)	Catalyzes the side chain degradation of 2,4-D herbicide
Dicamba tolerance	*Dmo*	*Stenotrophom-onas maltophilia* strain DI-6	Dicamba mono-oxygenase	Confers tolerance to the herbicide dicamba (2-methoxy-3,6-dichlorobenzoic acid) by using dicamba as substrate in an enzymatic reaction

Trait	Gene introduced	Gene source	Enzyme	Function
Sulfonyl-urea herbicide tolerance	*Zm-hra*	*Zea mays*	Herbicide tolerant acetolactate synthase (ALS)	Confers tolerance to sulfonylurea herbicides and other acetolactate synthase (ALS)-inhibiting herbicides
Isoxaflutole tolerance	*hppdPF W336*	*Pseudomonas fluorescens* strain A32	Modified p-hydroxyphenylpyruvate dioxygenase (HPPD)	Confers tolerance to HPPD-inhibiting herbicides (such as isoxaflutole) by reducing the specificity for the herbicide's bioactive constituent
Mesotrione tolerance	*Avhppd-03*	Oat *(Avena sativa)*	Modified p-hydroxyphenylpyruvate dioxygenase	Confers tolerance to HPPD-inhibiting herbicides (such as mesotrione) by reducing the specificity for the herbicide's bioactive constituent
Oxynil herbicides tolerance	*Bxn*	*Klebsiella pneumoniae* subsp. *ozaenae*	Nitrilase	The enzyme hydrolyses oxynil to non-phytotoxic compounds

3.2.3 Patterns of herbicide use

The introduction of GMHT crops has influenced the use pattern of herbicides. In general, a fairly broad range of mostly selective (grass weed and broad-leaved weed) herbicides has been replaced by broad-spectrum herbicides (mostly glyphosate) used in conjunction with one or two other (complementary) herbicides (Brookes and Barfoot, 2017). Globally, glyphosate use has risen almost 15-fold since the so-called "Roundup Ready," genetically engineered glyphosate tolerant crops were introduced in 1996 (Benbrook, 2012; Benbrook, 2016). At the same time, the use rates of some other herbicides have been reduced (Brookes and Barfoot, 2017; Kniss, 2017). Even so, some recent studies have shown that the total amount of herbicides applied in the main GM crops maize and soybeans has increased during the two decades since GM crops were introduced. However, as pointed out by (Brookes, 2014) and (Kniss, 2017), the amount of herbicides has increased also in conventional crops during the same period. A study based on farm level data from USA for the period 1998-2011 showed an increase in total herbicide use on an area basis in soybean while the use in maize decreased (Perry et al., 2016). A similar study of canola in Canada showed that the amount of active herbicide ingredient per hectare dropped substantially between 1995, prior to the commercialisation of GMHT canola, and 2006 when the adoption of GMHT canola was 95% (Smyth et al., 2011).

(Klümper and Qaim, 2014) who performed a meta-analysis of the impacts of genetically modified crops concluded that GMHT crops had reduced herbicide quantity in some situations, but have contributed to increases in the use of broad-spectrum herbicides elsewhere.

Several studies of effects of the introduction of GMHT crops on the use pattern have focused on the environmental impact rather than the total weight of various herbicides applied on the crops. The most common approach is to use an environmental impact quotient (EIQ) as a benchmark for environmental hazard. EIQs convert an array of attributes specific to each pesticide into a single value meant to summarise the potential toxicity of the chemical. Although the studies of pesticide use in GMHT crops give deviating pictures with respect to the trends in total herbicide use, most studies indicate a decrease in EIQ as a result of adoption of herbicide tolerant soybean and maize (Brimner et al., 2005; Brookes and Barfoot, 2017; Kleter et al., 2007; Perry et al., 2016).

This opinion focuses on the possible differences in pesticide residues in GMHT crops as compared to conventional crops at the time of harvest. The residue levels will depend on area loads and time of application of herbicides. Environmental impact of pesticides is not assessed in this opinion.

The increased use of GMHT crops has facilitated an expansion in total acres dedicated to conservation tillage, reducing the need for mechanical weed control. According to USDA Agricultural Resource Management Survey (ARMS) data, conservation tillage ranging from no-till to reduced-till was used on 75% of planted maize acres in 2010 (USDA-APHIS, 2014).

While the application of conservation tillage was only slightly higher in GMHT maize than in conventional varieties, there was a large difference among growers of soybeans. Eighty-five % of the adopters of GMHT crops used conservation tillage and only 35% of the growers of conventional soybean varieties in USA (Fernandez-Cornejo et al., 2014). Likewise, over half of the respondents in a survey among producers of GMHT canola in Canada indicated that they no longer use tillage operations in their cropping system (Smyth et al., 2010).

Development of resistance to herbicides in weeds is a major concern in agriculture. Herbicide resistance is the inherited ability of a plant to survive and reproduce following exposure to a dose of a herbicide normally lethal to the wild-type. Repeated use of a herbicide selects for herbicide resistant biotypes. Over time, the number of resistant individuals in the weed population increases until the majority of the population is herbicide resistant. The resistance is specific for the mode or site of action of the herbicide to which the weed population has been exposed. Herbicides employing a number of different modes and sites of action have been developed over the years. A recent review shows that weeds have evolved resistance to 23 of the 26 known herbicide sites of action and to 163 different herbicides (Heap, 2018).

Glyphosate was first marketed in 1974 but it was not until 1996 that the first occurrence of a weed resistant to glyphosate was reported. In the following years, the number of weed species that evolved resistance to glyphosate increased to more than 40, distributed across 37 countries (Heap, 2018; Heap and Duke, 2017) (Figure 15). During the same period, the amount of glyphosate used globally increased almost 15-fold to reach 826 000 tons in 2014, mainly driven by the introduction of glyphosate tolerant crops (Benbrook, 2016; Duke, 2017).

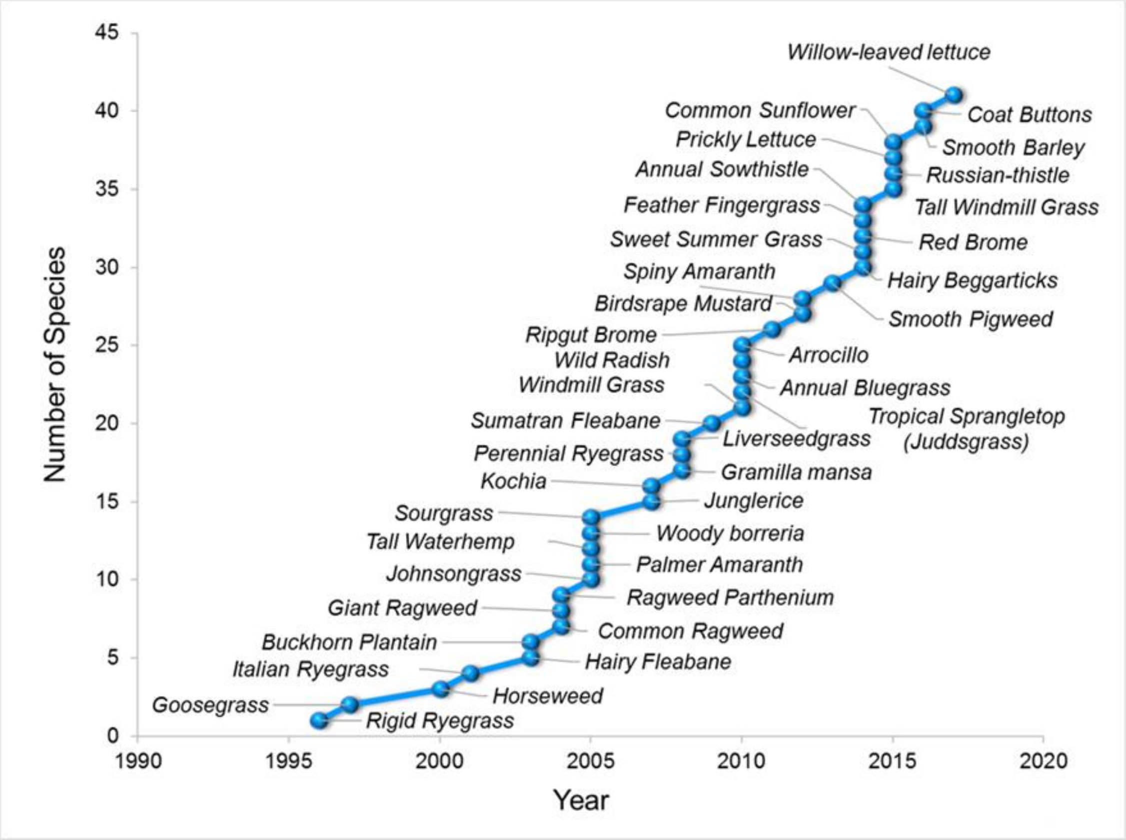


Figure 15. Increase in glyphosate resistant weeds worldwide (Heap, 2018).

Because of the increasing challenges with glyphosate resistant weeds, farmers are now advised to include other herbicides (with different and complementary modes of action) in combination with glyphosate and in some cases to adopt cultural practices (e.g., revert to ploughing) in more integrated weed management systems (Brookes and Barfoot, 2017). This has reversed the initial trend of reduced volumes of herbicides in certain GMHT crops and regions (Bonny, 2016). Data from the United States Department of Agriculture's National Agricultural Statistical Service (USDA-NASS, 2018) reflects this trend for herbicide use in soybeans. The use of glyphosate measured in kg per planted hectar increased proportionally to the adoption rate of glyphosate tolerant soybean since 1996. The use of herbicides other than glyphosate declined to a minimum in 2005 and has increased during the last decade (Figure 16). This increase cannot be explained by the subsequent expansion of planted area, which was approximately 16%.

When GMHT maize was introduced on the market in USA in 1998, it was not adopted by farmers as rapidly as GMHT soybean. GMHT maize increased from 7% of maize acres in 2000 to 26% in 2005 and 85% in 2013 (Fernandez-Cornejo et al., 2014). This is reflected by the use of glyphosate in maize, which remained low during the first years after the introduction of glyphosate tolerant maize (Young, 2006), but increased after 2001 (Figure 16).

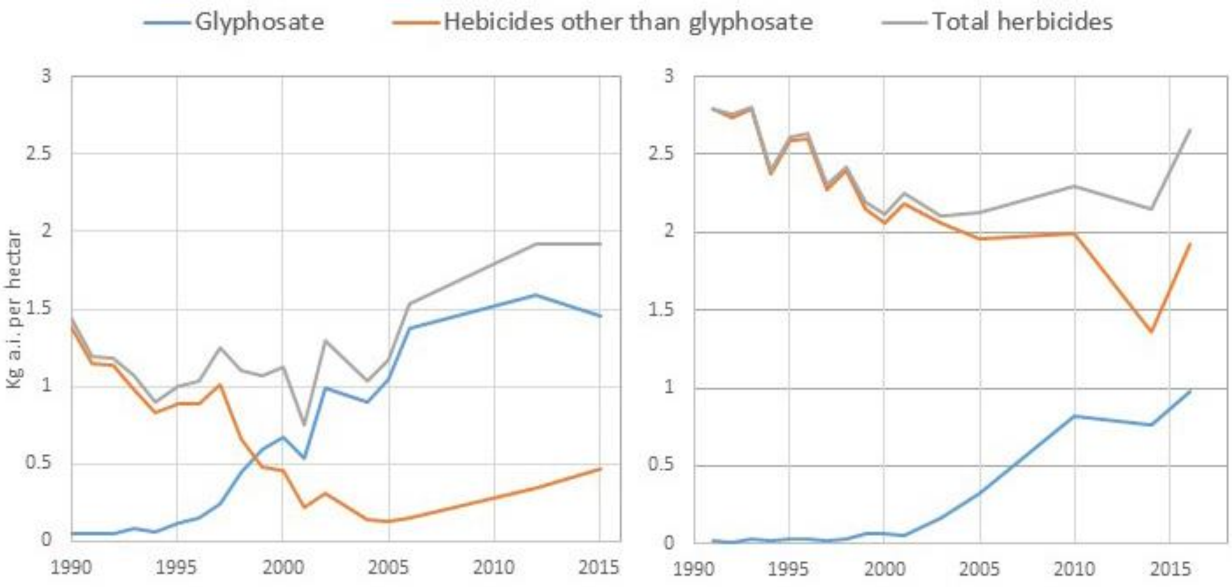


Figure 16. Area use of glyphosate and other herbicides used in soybeans (left) and maize (right) in USA in the period 1991-2016, according to data from the (USDA-NASS, 2018).

On a volume basis, glyphosate contributed less than 5% of total herbicides applied in maize and soybeans in the early 1990's. In 2015, glyphosate constituted 76% of herbicides applied in soybeans and 37% in maize (Figure 17).

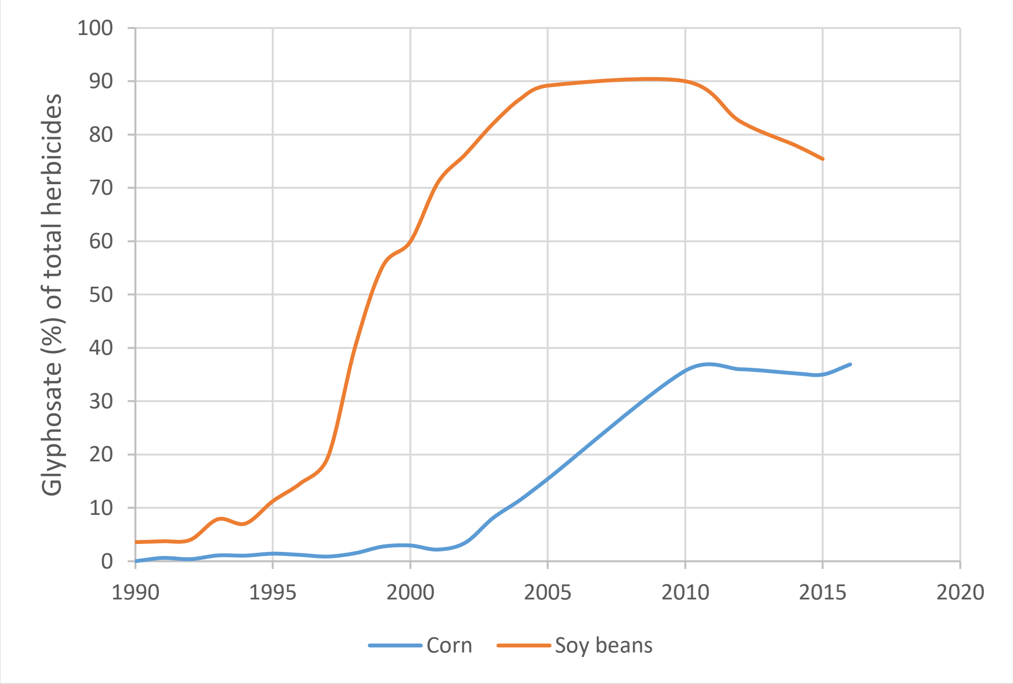


Figure 17. Glyphosate in percentage of total herbicide applications in soybean and maize in USA 1990-2016 (adopted from USDA-NASS, 2018).

The changes in pesticide use shown in the USA national database (USDA-NASS, 2018) during the last 30 years is a strong indication of the effects of introduction of GMHT crops of soybean and maize. However, comparing the present use pattern when more than 90% of these crops are GMHT, with the herbicide use in 1995 does not give a correct picture of the difference between GMHT and conventional production systems today for several reasons: (i) The herbicides available change because of registration/deregistration, (ii) the focus on conservation tillage practices increases the need for herbicides, and (iii) the increased occurrence of herbicide-resistant weeds calls for more diverse application of herbicides.

Few studies have been done to compare the actual use patterns of herbicides in GMHT- and conventional agriculture. Perry and colleagues (Perry et al., 2016), analysed farm-level data collected over the period 1998-2011 in USA. They found that for both soybeans and maize there has been a significant increase in non-glyphosate herbicides applied by adopters of glyphosate tolerant crops. In soybeans, an adopter of glyphosate tolerant crops in 1998 used about 0.71 kg/ha less non-glyphosate herbicides relative to a conventional user; by 2011, the difference was just 0.48 kg/ha. In maize, adopters of glyphosate tolerant crops went from using 1.31 kg/ha less of non-glyphosate herbicides in 1998 to only 0.32 kg/ha less in 2011.

Fernandez-Cornejo and colleagues (Fernandez-Cornejo et al., 2014), reported higher total herbicide use in GMHT soybean (1.52 kg/ha) than in conventional soybean (1.18 kg/ha), based on data from a survey in USA from 2006. For maize, however, total herbicide use was lower in GMHT (1.7 kg/ha) than in conventional (2.6 kg/ha) in 2005. Five years later, the areal herbicide application in both categories was approximately 2.4 kg/ha. The authors suggested that the increased herbicide use in GMHT maize in recent years was due to herbicide resistant weeds. They concluded that despite the mixed but relatively minor effect GMHT crop adoption has had on overall herbicide usage, the main effect of GMHT crop adoption was the conversion to primarily use glyphosate based herbicides and proportionally less traditional herbicides.

For Argentina and Brazil, the available data on pesticide use is less detailed. The Food and Agriculture Organization of the United Nations (FAO) has published data on total herbicides used per year, however, without specification of active ingredients or crops (FAOSTAT, 2018). In both countries, the use of herbicides has increased approximately a factor 10 since the beginning of the 1990-ies and reached 194 000 tons in Argentina and 216 000 tons in Brazil in 2014. During the same period, the area of the main agricultural crops has increased approximately by a factor of two in both countries, mainly due to the rapid rise in soybean cultivation after introduction of GM soybeans (Figure 18). In Brazil, GMHT soybean was authorised in 2003. In the following 10 years, the use of herbicides in soybean increased 175% according to data reported by Almeida and colleagues (Almeida et al., 2017). The corresponding increase in area of soybean cultivation was 52% (FAOSTAT, 2018). Thus, it appears that a substantial increase in the area load of herbicides in Argentina and Brazil has happened after the introduction of GM crops. Some reports indicate that glyphosate use rates in soybean in Brazil and Argentina can be much higher than in USA (Benbrook, 2016).

According to Meyer and Cederberg (Meyer and Cederberg, 2010), the average use rate in Brazilian soybean increased from 2.8 kg/ha in 2003 to 4.2 kg/ha in 2008.

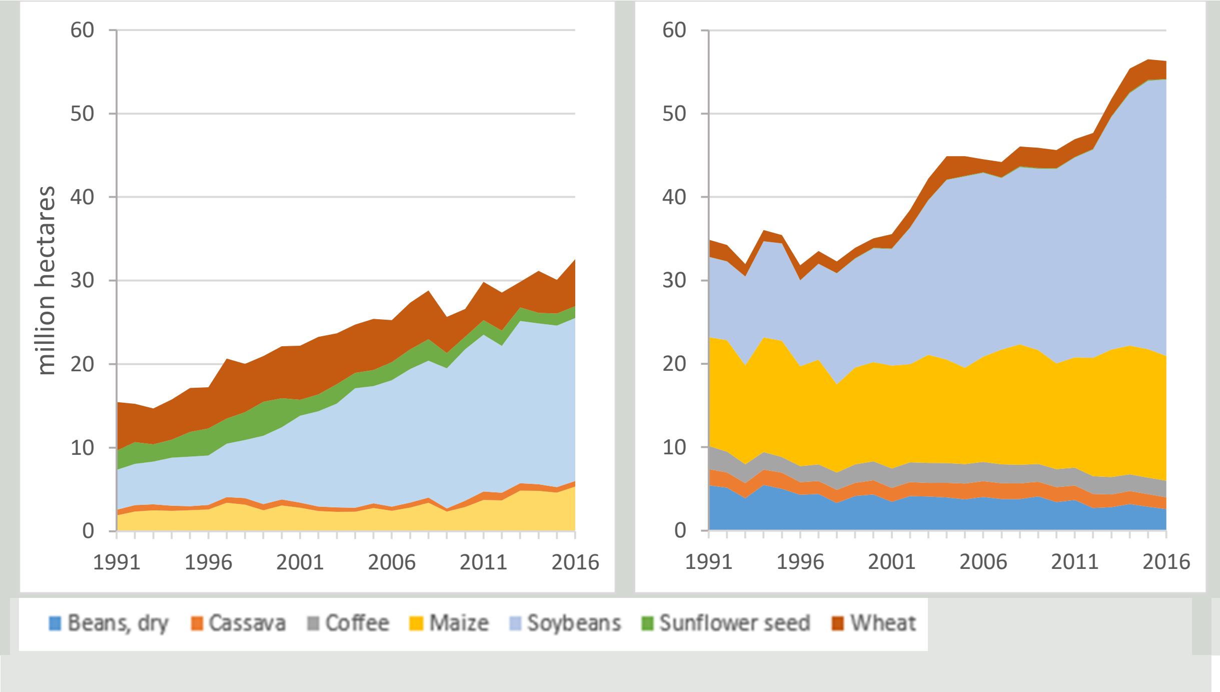


Figure 18. Area harvested in Argentina (left), and Brazil (right) 1991-2016 (adopted from FAOSTAT, 2018).

In 2017, the adoption rate of GMHT crops in Brazil was 97% for soybean and 90% for maize. In Argentina, essentially 100% of soybean and 97% of maize were GMHT crops (ISAAA, 2017).

In order to assess the potential for residues of pesticides in the crops at harvest, not only the application rate, but also factors like time of application as well as uptake, distribution and degradation in the plants are of importance.

Before the introduction of glyphosate tolerant crops, the main agricultural use of glyphosate was as a non-selective herbicide for pre-plant burndown of weeds (Duke, 2017). Glyphosate adsorbs rapidly and binds strongly to most soils which limits the uptake in plants via the roots. Therefore, uptake of glyphosate remaining in the soil after pre-planting application is normally insignificant in the following crop (EFSA, 2015).

An additional use of glyphosate in conventional agriculture is as a desiccation agent. This practice involves pre-harvest application of glyphosate on the standing crop and is used in cereals and oilseed crops. Pre-harvest glyphosate may also be used to control perennial weeds. In Norway glyphosate is approved for control of couch grass (*Elytrigia repens*) in ripe barley, with a seven-day withholding period.

When sprayed on the green foliage, glyphosate is actively taken up by the plants and translocated to the meristematic tissue in root and shoot apices.

The timing of application is crucial as the moisture content of the grain must be below 30% for the yield and quality of the crop to be unaffected, and to minimize residue levels of glyphosate in the grain (Zhang et al., 2016). Nevertheless, the practice of pre-harvest application as a harvest aid is likely to cause higher residue levels of glyphosate in the harvested foodstuffs. To cover such residues, increased tolerances for GMHT crops have generally been granted in USA. For instance, the tolerance levels for glyphosate in soybean grain, hay and forage in 1993 were 20, 15 and 15 mg/kg, respectively, whereas they in 2015 were 40, 100 and 100 mg/kg, respectively (Benbrook, 2016).

In glyphosate tolerant crops, glyphosate is applied on the culture after emergence of the plants and is actively taken up by the plants. The residue levels at harvest will depend on the time of application and the degradation rate of glyphosate in the plants. Thus, the levels of residues are likely to be higher than with the normal conventional practice where glyphosate is used before planting or after harvest.

3.3 Summary

Maize, soybean and oilseed rape are the main herbicide tolerant edible crops in terms of global production volumes. To answer ToR 1, the agricultural practices involved in the production of conventional and herbicide tolerant varieties of these crops are described, with focus on weed management strategies. Very little information is available for these crops in Norway and/or the rest of Europe. Description of agricultural practices is mainly based on information from USA, South America and Australia.

GM technology has been widely adopted in agriculture since the first GM crops were marketed in the mid-1990s. In 2017 approximately 190 million hectares of biotech crops were planted by farmers in 24 countries.

The most adopted trait that has been introduced in GM crops is tolerance to herbicides and in particular to glyphosate. In many countries, GMHT varieties have almost completely replaced conventional varieties of maize, soybean and oilseed rape.

The shift from conventional to GMHT crops has implied significant changes in agricultural practices and in particular the weed management strategies. In conventional crops, broad-spectrum herbicides, such as glyphosate, are mainly used for burndown of weeds after harvest or before planting, although glyphosate may also be used as a desiccating agent before harvest in some crops. In glyphosate tolerant crops, glyphosate can also be applied to control weeds after emergence of the crops, which reduces the need for use of other more selective herbicides. Furthermore, the introduction of GMHT crops has contributed to the adoption of no-till farming.

The effect of the increasing adoption of GMHT crops is reflected in the user statistics of herbicides in USA, where such crops have become dominating. The most obvious trend in use of herbicides is the extensive increase in use of glyphosate, which has occurred along with the adoption of glyphosate tolerant varieties of soybean and maize. On a volume basis glyphosate contributed less than 5% of total herbicides used in maize and soybean cultivation in the early 1990's. In 2015, when the adoption rate of GMHT varieties had reached more than 90%, glyphosate constituted 76% of herbicides applied on soybeans and 37% on maize. Globally, the use of glyphosate increased almost 15-fold in twenty years, to reach 826 000 tons in 2014. In some countries in South America, there has been an approximately five-fold increase in the area load of herbicides after the introduction of GM crops.

The area loads of herbicides other than glyphosate on soybean and maize initially declined after introduction of glyphosate tolerant varieties, but this trend has seemingly been reversed during the last decade.

Comparative studies of herbicide use have shown deviating results in the total amounts of herbicides applied in GMHT- and conventional cultivation of the same crop. For crops grown in North America, GMHT adoption has reduced the use of herbicides on maize and oilseed rape (canola), while the use on soybean has increased. However, several studies have shown an increased use of non-glyphosate herbicides in glyphosate tolerant crops in recent years. This trend has been linked to the development of glyphosate resistant weeds, which has happened after the introduction of glyphosate tolerant crops. Today, more than 40 species of weeds have developed resistance to glyphosate, and farmers must therefore include herbicides other than glyphosate in order to combat such weeds.

The differences in patterns of herbicide use between conventional and GMHT crops are likely to have effects on the level and composition of residues of herbicides in the harvested crops. The many fold increase in the area load of glyphosate in glyphosate tolerant crops deserves special attention in this respect. For herbicides other than glyphosate, the loads may be expected to be lower in glyphosate tolerant than in conventional crops. However, due to the increasing occurrence of glyphosate resistant weeds, the use of such herbicides has increased in glyphosate tolerant crops in recent years.

4 Residue levels of glyphosate, its metabolites and other relevant herbicides used on glyphosate tolerant and conventional crops (answer to ToR 2)

4.1 Glyphosate residues and metabolites in GM glyphosate tolerant maize, soybean and oilseed rape, and in their conventional counterparts

A maximum residue level (MRL) is the highest level of a pesticide residue that is legally tolerated in or on food or feed when pesticides are applied correctly according to good agricultural practice (GAP). Several considerations must be taken into account when the MRL is set. These include information on the use of the pesticide on a crop, e.g. application rates and time of application, experimental data on expected residues and toxicological reference values for the pesticide. Based on available information, the intake of residues through all food that may be treated with the given pesticide is compared with the Acceptable Daily Intake (ADI) and Acute Reference Dose (ARfD) for all consumers, including vulnerable groups, to ensure consumer safety.

At present, the permitted MRL for glyphosate is the same for GM and conventional crops in the EU (equally to other pesticides). In the EU, the MRL for glyphosate is 20 mg/kg for soybean, 1 mg/kg for maize (3 mg/kg for sweet corn) and 10 mg/kg for oilseed rape (EU, 2013).

Upon request from the EU Commission, EFSA recently performed a review of the existing MRLs for glyphosate (EFSA, 2018b). EFSA operates with one main and one optional definition of glyphosate residues as basis for the review to help national authorities (risk managers) in the EU to control and ensure that MRLs are not exceeded in products on the EU marked:

I) “for all plant commodities, including plants with glyphosate tolerant genetically modified varieties currently available on the market: sum of glyphosate, AMPA and N-acetyl-glyphosate, expressed as glyphosate” (optional definition)

and

II) "for plants with glyphosate tolerant genetically modified varieties currently available on the market (sweet corn, cotton seeds, sugar beets, rapeseeds, maize and soybeans): sum of glyphosate, AMPA and N-acetyl-glyphosate, expressed as glyphosate;
- for all other plant commodities: glyphosate." (Main definition).

The report further specifies that:

"For risk assessment, a general residue definition covering both conventional and genetically modified crops was proposed as the sum of glyphosate, AMPA, *N*-acetyl-glyphosate and N-acetyl-AMPA, expressed as glyphosate."

The report concludes regarding the residues in all assessed conventional crops, that the available data are considered sufficient to derive (tentative) MRL proposals as well as risk assessment values except for a list of some commodities, including soybeans and maize straw. For these, the available data were insufficient to derive MRLs and risk assessment values. For GM crops, the report concludes that data were sufficient to derive MRLs for sweet corn (EPSPS modification), noting that MRLs should be tentative pending on the submission of confirmatory methods for enforcement of AMPA and *N*-acetyl-glyphosate. For maize and soybeans (EPSPS modification), soybeans (GAT modification) and rapeseeds (GOX modification), the available data were insufficient to derive MRLs and risk assessment values.

4.1.1 Data from research articles

The literature searches described in chapter 2.2 for data on residue levels of glyphosate or its metabolites in maize, soybean or oilseed rape retrieved 546 titles, but only 14 papers were found that actually contained data on levels of residues in plants (or plant material) after spraying with glyphosate. Least data were found for oilseed rape (2 papers), while we found 6 and 8 papers for maize and soybean, respectively[1]. All in all, the data were very heterogeneous. Residues were measured in different parts of the plants (leaf, stem, seed etc.), and glyphosate was applied at different rates and at different time points during the season. In addition, the time points for the sampling varied.

Of the 14 papers containing data on residue levels, 10 reported data from their own original analyses. Seven of these reported data from analyses of samples from field experiments, one referred to analysis of plants that have been sprayed at «rates and times that are usually used by the farmers in accord with agricultural practices proposed by technical advisers», while only two had measured residue levels of glyphosate in samples from crops that were

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[1] Some papers contain data for more than one of the commodities, and these numbers also include the review, therefore the sum exceeds 14.

actually intended for use as food or feed (one of these two have analysed soybean protein concentrate).

Seven papers reported data on residue levels in soybean, four of these had measured the residue concentrations in the actual bean (Arregui et al., 2004; Bohm et al., 2008; Bøhn et al., 2014; Duke et al., 2003). The other three had measured residues in soybean protein, immature beans, or stem and leaf (Ehling and Reddy, 2015; Lorenzatti et al., 2004; Reddy et al., 2008). The four studies reporting measurements in the bean, reported residues of both glyphosate and AMPA. All four studies had measured residues in glyphosate tolerant GM soybean. Bohm and colleagues (Bohm et al., 2008) and Bøhn and colleagues (Bøhn et al., 2014) have measured residues of glyphosate and AMPA in both conventional and GM soybean. The conventional soybeans had not been treated with glyphosate, and no residues of glyphosate was detected in these samples.

The highest concentration reported within each of the four studies on soybean varied from 1.8 mg/kg (Arregui et al., 2004) to 36 mg/kg (Bohm et al., 2008) for glyphosate, and from 0.9 mg/kg (Arregui et al., 2004) to 25 mg/kg for AMPA (Duke et al., 2003).

The highest levels of glyphosate residues in GM soybean were reported from Brazil. Here, 960 g/ha glyphosate (brand/producer unknown) was applied. Treatment was carried out 28 and 56 days after planting and the beans were harvested 147 days after planting (Bohm et al., 2008). The reported value of 36 mg/kg is above the current MRL for glyphosate in soybean in the EU (20 mg/kg). However, this single value is not supported by other publications with similar treatments.

The study by (Arregui et al., 2004), reporting the lowest levels of both glyphosate and AMPA residues in GM soybeans, was from Argentina. In this study glyphosate (Roundup[2]) was applied at the same rate (960 g/ha) at 9, 84 and 94 days after planting. These beans were harvested 133 days after planting.

Only one of five papers on residue levels in maize referred to levels in maize grain. This paper did not report their own measurements, but only referred to glyphosate residues detected in supervised trials reviewed by EU under the directive concerning the placing of plant protection products on the market. They reported min. <0.05 mg/kg, max. 2.6 mg/kg and median 0.1 mg/kg (Gaston and Harris, 2004). In the EU, the MRL for maize is 1 mg/kg (EU, 2019a).

The other papers reported levels in leaf, apex, stem and/or root (Bernal del Nozal et al., 2012; Doublet et al., 2009; Oulkar et al., 2017; Reddy et al., 2008). For maize silage, residues of other parts of the plant than grain are obviously also relevant.

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[2] Glyphosate-isopropylamine 480 g AE litre−1 SL.

The highest levels of glyphosate residues were reported from Spain, with 1.6 kg/ha in shoot 0 days after treatment (Bernal del Nozal et al., 2012). The level of glyphosate residues in these shoots was 8.91 mg/kg, and the level of AMPA residues was 0.16 mg/kg. At 56 days after treatment however, the level of glyphosate was 0.01 mg/kg, and the level of AMPA was <LOD. These residue levels were measured in GM plants.

Only two papers reported levels of glyphosate in oilseed rape. One of these papers focused on degradation in soil of glyphosate previously absorbed by plants (Doublet et al., 2009). They reported 69% absorption of glyphosate (Roundup) in the whole plant 7 days after treatment. In this study, droplets of glyphosate were applied directly onto the second youngest leaf of the oilseed rape plants. The other paper was an opinion from EFSA on the import tolerance for glyphosate in genetically modified oilseed rape (EFSA, 2013). Here, trials using Canada GAP[3] and USA GAP[4] are reported. Fifteen residue trials complied with the authorized Canadian GAP. Residues of AMPA ranged from below the LOQ of 0.05 mg/kg up to 0.082 mg/kg, residues of N-acetyl-AMPA were at or below 0.05 mg/kg in all, except one sample which containing 0.34 mg/kg, glyphosate ranged from 0.41 mg/kg to 8.95 mg/kg and N-acetyl-glyphosate ranged from 0.23 mg/kg to 3.15 mg/kg with an exception of one sample with 14 mg/kg. Seven residue trials were compliant with the USA GAP. Residues of AMPA were in all samples below the LOQ of 0.05 mg/kg, residues of glyphosate were below the LOQ of 0.05 mg/kg in all samples, except one with 0.365 mg/kg, N-acetyl-glyphosate ranged from 0.076 mg/kg to 1.9 mg/kg and N-acetyl-AMPA ranged from <0.05 mg/kg to 0.55 mg/kg.

4.1.2 Data from monitoring programmes

Pesticide residue monitoring programmes in Norway, EU, USA and Australia were checked in the search for data on residue levels of glyphosate and its metabolites, and for residue levels of the 10 most frequently used herbicides in conventional and GM crops (see chapter 3.2). The newest published data were used, and for some countries reports from several years were examined. The oldest data used were from 2011.

Very little data for comparison of residue levels in GM versus conventional crops could be collected from the monitoring reports that were available (i.e. publicly accessible and in English or a Scandinavian language). In Norway, rye and barley were the only food commodities in which glyphosate residues were analysed, in addition to soybean and oilseed rape for feed. However, Norway has not authorised any GM crops for import and food and feed uses and hence would not have any data on residues in GMHT crops. Also in the EU, very few samples of maize or soybean have been analysed for residues of glyphosate (2015;

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[3] 0.68 kg active substance (a.s.)/ha at pre-emergence (BBCH 11-16) + 0.68 kg a.s./ha (at 6-leaf stage) + 0.9 kg a.s./ha at pre-harvest (PHI 7 days).

[4] 1.8 kg a.s./ha at pre-emergence (BBCH 11-16) + 0.62 kg a.s./ha at the PHI of 60 days.

33 maize samples, 11 soybean samples. 2016; 38 soybean samples). Although glyphosate was detected in 16% of the soybean samples in 2016, no samples of neither maize nor soybean exceeded the MRL. In the total diet study from Australia (FSANZ, 2011), glyphosate was detected in multigrain bread (in very low concentrations), but no data were reported on maize, soybean or oilseed rape.

In USA, glyphosate has so far not been included in the annual monitoring programme for pesticide residues by the US Food and Drug Administration (FDA). However, in 2011 the US Department of Agriculture (USDA-AMS, 2011) included testing for glyphosate and its AMPA metabolite in 300 soybean samples in their Pesticide Data Program. Most of the samples (271; 90.3%) contained glyphosate at levels ranging from 0.26 mg/kg to 18.5 mg/kg. The AMPA metabolite was detected in 287 (95.7%) of the samples at levels ranging from 0.26 mg/kg to 20 mg/kg. It was not reported whether these soybean samples were GM or non-GM.

The data collected from publications found in the literature search and monitoring programs were insufficient for a systematic comparison of residue levels in GM versus conventional crops, although data from both glyphosate tolerant GM plants, and conventional plants were found.

4.2 Residue levels of other relevant herbicides used on glyphosate tolerant and conventional maize and soybean varieties

As described in chapter 3, weed control practices related to cultivation of GMHT crops have changed since the development and adaptation of glyphosate tolerant crops in food production. Whereas the first decade saw a significant decline in overall use of non-glyphosate herbicides in favour of glyphosate-based herbicides, the onset of glyphosate resistance in many important weeds have since forced farmers to adopt new weed management strategies. These new strategies include supplementing use of glyphosate with herbicides that have different modes of action.

In accordance with the project's ToR 2, the goal was to look for not only glyphosate residues, but also residues of other relevant herbicides used with both glyphosate tolerant crops as well as with conventional crops, and implicitly disclose whether or not there are significant differences or certain trends in residue levels between the two types of crops. For this purpose, the project sought to compare residue levels of frequently used herbicides in glyphosate tolerant and conventional varieties of maize and soybean, two of the major crops produced worldwide.

The selection of herbicides was based on surveys conducted in 2015 and 2016 for maize and soybean, respectively, from the Agricultural Chemical Use Program provided by the United States Department of Agriculture (USDA-NASS, 2018). The survey data do not distinguish between GM and conventional crops. No data on herbicide use was found for oilseed rape in the database, oilseed rape was therefore not further investigated for this part of ToR 2.

The top 10 herbicides were selected ranked by total annual tonnage used. Some major herbicides, e.g. glufosinate, 2.4-D, and dicamba, were excluded since both tolerant GM maize and/or soybeans have been developed for these herbicides.

The selected 10 herbicides that were further scrutinised for residue data are listed in Table 2.

Table 2. The 10 most applied conventional herbicides in soybean (7 herbicides) and maize (6 herbicides) production in USA in 2015 and 2016, respectively (adopted from USDA-NASS Database, March 2018).

Herbicide	**Soybean, metric tons of herbicide used in 2015**	**Maize, metric tons of herbicide used in 2016**
Atrazine	---	25409
Acetochlor	1026	16587
Clopyralid	---	411
Dimethenamid	688	883
(S-) Metolachlor	5856	14180
Metribuzin	833	---
Fomesafen	1435	---
Paraquat	693	---
Pendimehtalin	699	---
Simazine	---	582

Only one publication was obtained by the comprehensive literature search described in chapter 2.2.2. The publication describes the half-life and residues of S-metolachlor in maize seedlings and in soil. The half-life of S-metolachlor in maize seedlings in Beijing and Changchun were 6.68 and 4.85 days, respectively. Terminal residues in maize seeds were not detectable (Cao et al., 2008). The article does not state whether or not the maize used was GM or not.

All pesticides have a set maximum residue level (MRL) which is not to be exceeded in the harvested crops (or other commodities) whether a crop is GM or not. Food safety authorities such as the FDA, EFSA and the Norwegian Food Safety Authority (NFSA) continuously monitor and sample different food commodities to ensure that the pesticide MRLs are not exceeded, taking action when required, to protect the consumers. In an effort to obtain data to answer the second part of ToR 2, the project group searched the webpages of EFSA, FDA, FAO and NFSA for the 10 selected herbicides and reported measurements of residue levels in maize and/or soybean commodities. Although all 10 herbicides are measured and/or evaluated by one or several of the organisations listed, no relevant data was found that would enable the project group to distinguish any differences in residue levels between conventional and glyphosate tolerant GM maize and/or soybean crops for these 10 herbicides. None of the reports found specify whether or not the measured maize or soybean samples are from GM plants. Although an argument could be made that sampled maize and soybeans from certain countries, e.g. USA, most likely come from GM crops, and likewise that conventional crops are expected to come from certain other countries, the sum of the data we found is insufficient to make any meaningful comparisons between GM and conventional maize and soybean crops in regard to the questions in ToR 2.

4.3 Summary

There is very little data available on glyphosate residue levels, both in conventional and glyphosate tolerant crops, of maize, soybean and oilseed rape. The data collected from our literature searches were therefore insufficient for a systematic comparison of these crops. To answer the first part of ToR 2, fourteen research papers were found to include glyphosate residue levels, some also including metabolites. However, the data were heterogeneous and practically not comparable. Residues were measured in different parts of the plants (leaf, stem, seed etc.), application rates varied as did seasonal treatment and sampling times.

In accordance with the project's second part of ToR 2, residues of other relevant herbicides used with both glyphosate tolerant crops as well as with conventional crops, were also to be investigated. For this purpose, the project group sought to compare residue levels of 10 selected herbicides in maize and soybean. The 10 herbicides were selected based on their total annual use in USA (in tons) according to surveys conducted in 2015 and 2016 for maize and soybean, respectively. However, the literature searches returned no relevant hits. In addition, since the available survey and monitoring data did not distinguish between GM and conventional crops, it was not possible to conclude whether these two types of crops differed regarding residue levels of the 10 selected herbicides.

5 A description of how the genetic modifications used to make a plant tolerant against glyphosate may influence the metabolism of glyphosate or other plant protection products (PPPs) (answer to ToR 3)

As mentioned in chapter 3.2.2, there are two main strategies to introduce glyphosate tolerance into crop species. One, and by far the most utilised, is the insertion of a bacterial gene encoding an EPSPS enzyme with low affinity for glyphosate, e.g. CP4 EPSPS. The second is insertion of bacterial genes encoding enzymes, e.g. GAT and GOX, that react with and detoxifies glyphosate directly.

The *cp4 epsps* gene from *Agrobacterium tumefaciens* strain CP4 was the first to be utilised in development of a GMHT soybean, and is still the most utilised single trait in important GM crops such as maize, soybean and oilseed rape.

Traits based on metabolic inactivation of glyphosate utilises either the *goxv247* gene from *Ochrobactrum anthropi* strain LBAA, or the *gat* gene from *Bacillus licheniformis*, encoding the enzymes glyphosate oxidoreductase (GOX) and glyphosate *N*-acetyl transferase (GAT), respectively.

The GOX enzyme cleaves the nitrogen-carbon bond in glyphosate converting it to glyoxylate and aminomethylphosphonic acid (AMPA). GAT catalyses acetylation of glyphosate subsequently producing *N*-acetyl-glyphosate which has no herbicidal activity. The use of GAT also leads to production of *N*-acetyl-AMPA in the transformed plants (EFSA, 2018a; EFSA, 2018b).

In GM crops that are tolerant to glyphosate due to CP4 EPSPS or other modified versions of the enzyme, changes in glyphosate metabolites are not expected to be different from those found in conventional crops, since the function of CP4 EPSPS is to drive the biosynthesis of aromatic amino acids and not to interact with glyphosate. In crops with the GOX enzyme, the types of metabolites are also expected to be the same as in conventional crops, however, the balance between glyphosate and enzyme reaction products is more skewed towards the products (metabolites) compared to in conventional crops. Only the GAT enzyme introduces different types of metabolites in the GM crops compared to conventional crops (and genetically modified crops expressing the CP4 EPSPS and GOX enzymes), due to the enzymatic acetylation of glyphosate (EFSA, 2018a; EFSA, 2018b). The major enzymatic products and therefore the major metabolites in plants expressing GAT is *N*-acetyl-glyphosate (Figure 19), and to a lesser degree *N*-acetyl-AMPA.

Thus, this degradation results in four residues in the plants and in the environment; glyphosate, *N*-acetyl-glyphosate, AMPA and *N*-acetyl-AMPA.

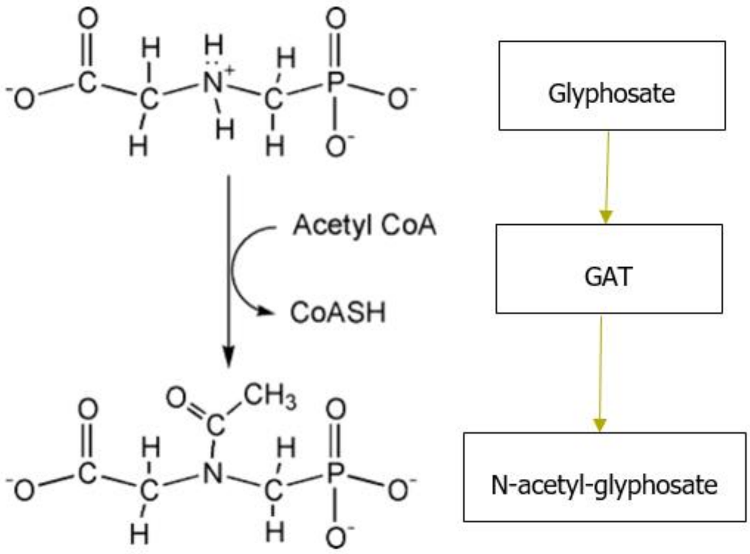

Figure 19. Acetylation of glyphosate by glyphosate *N*-acetyl transferase (GAT).

In order to answer ToR 3 on whether or not genetic modifications, introducing GAT or GOX, can alter the metabolism of other plant protection products (PPPs), two approaches were used.

The first approach was to perform literature searches for published papers studying any of 15 selected PPPs (herbicides, fungicides and insecticides) likely to be used on glyphosate tolerant crops. The list of 15 PPPs was set up based on available data on their highest annual use in USA (in tons) for maize and soybean in the period 2012 – 2016 (Table 3) (USDA-NASS, 2018). No data on use were found for oilseed rape in this database.

The second approach was to see if any of the 15 PPPs could function as substrates for the two enzymes glyphosate *N*-acetyltransferase (GAT) or glyphosate oxidoreductase (GOX).

Table 3. A list of 15 PPPs (pesticides) that may be used on maize or soybean that have been modified to enzymatically detoxify glyphosate. Pesticides were chosen based on their annual use (in tons) on maize or soya during the period 2012 - 2016 (adopted from USDA-NASS, March 2018).

The most used pesticides in maize cultivation in USA, 2014-2016				**The most used pesticides in soybean cultivation in USA, 2012-2015**		
Herbicides	**Tons/year**		**kg/ha**	**Tons/year**		**kg/ha**
Glyphosate	*37314*		*1,48*	*49594*		*1,81*
Atrazine	25409		1.22	-		-
Acetochlor	17587		1,53	-		-
Metolachlor	14180		1,66	5856		1,49
Mesotrione	1280		0,13	-		-
2,4-D	1180		0,26	3471		0,60
Dicamba	1072		0,22	-		-
Fungicides	**Tons/year**		**kg/ha**	**Tons/year**		**kg/ha**
Pyraclostrobin	250		0,14	180		0,11
Propiconazole	129		0,09	141		0,13
Azoxystrobin	107		0,09	169		0,14
Insecticides	**Tons/year**		**kg/ha**	**Tons/year**		**kg/ha**
Propargit	391		2,21	-		-
Acephate	---		---	448		1,07
Bifentrin	120		0,08	-		-
Chlorpyrifos	105		0,73	947		0.50
Dimethoate	-		-	125		0,52
λ-cyhalothrin	-		-	95		0,035

The initial comprehensive literature searches returned no relevant articles with either of the enzymes GAT or GOX when combined with any of the 15 PPPs listed in Table 3.

However, three articles were found relevant to the topic(s) of ToR 3. All three were written by the developers of the GAT enzyme, Pioneer Hi-Bred International. The articles described the discovery and focused development of the initial enzyme through multiple modifications made to the encoding bacterial genes finally resulting in the GAT-version with high specificity and affinity towards glyphosate (Castle et al., 2004; Siehl et al., 2005; Siehl et al., 2007).

The evolved GAT enzyme is an *N*-acetyl transferase from *Bacillus licheniformis* that has been optimized by gene shuffling for acetylation of glyphosate, forming the basis for a novel mechanism of glyphosate tolerance in transgenic plants (Siehl et al., 2007). The structure-activity data on this enzyme indicated a narrow substrate range for native GAT and variants optimised for acetylation of glyphosate (Siehl et al., 2005; Siehl et al., 2007). According to the developers, appreciable activity required an amine-containing compound with a phosphonyl or phosphoryl group and a carboxyl group with a main chain length of five or fewer atoms. Of the numerous compounds that were tested, those that laid outside these parameters all failed to exhibit appreciable activity with native GAT or any variant (Siehl et al., 2007).

Of the 15 herbicides, fungicides and insecticides listed in Table 3 that are likely to be used on GM maize or soybean with GAT activity, only dimethoate and acephate appear to have a chain length of five or fewer atoms, and to have a carbonyl (modified carboxyl) group, an amino group and a P-containing group (Figure 20). However, in order to ascertain whether these compounds could be metabolised by GAT, further studies into pesticide-enzyme binding and the kinetics of such a reaction would be needed. This view has been further corroborated by Professor Trond Vidar Hansen at the University of Oslo, School of Pharmacy (personal communication). With regard to the possibility of this enzyme to acetylate the other 15 pesticides, he stated: "*The answer to this for the 15 specified chemicals I would say is no/to a very limited degree. However, the word react is a broad term, so one would have to do experiments in order to find out if glyphosate acetyl transferase could induce reactions other than acetylation, such as hydrolysis, N-dealkylation or cause allosteric effects.*"

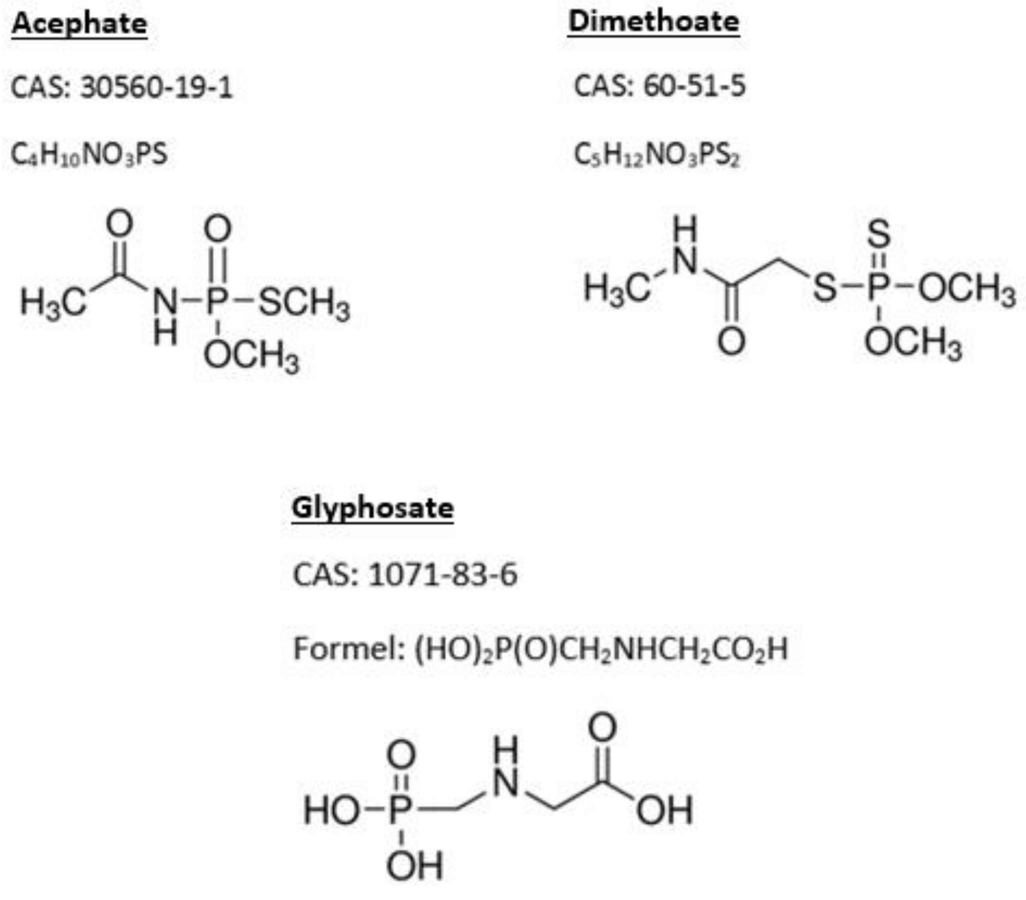

Figure 20. Chemical formula and molecular structures of acephate, dimethoate and glyphosate. Although structurally related only glyphosate is expected to be a good substrate for glyphosate *N*-acetyl transferase (GAT).

5.1 Summary

The literature searches returned no relevant articles to answer ToR 3. Based on structure-activity studies and expert judgement, it is considered unlikely that any of the 15 herbicides, fungicides and insecticides that may be used on GM maize or soybean with GAT activity would function as substrate for the GAT enzyme. However, experimental data are needed to answer this part of the ToR with more certainty.

6 An evaluation of whether possible changes in the spectrum of metabolites may have implications for the toxicity of glyphosate tolerant crops (answer to ToR 4)

In GM crops that are tolerant to glyphosate due to expression of modified versions of EPSPS, a similar glyphosate metabolite spectrum to that found in conventional crops is expected. In crops with the GOX enzyme, the types of metabolites are also expected to be the same as in conventional crops, however, the balance between glyphosate and enzyme reaction products is more skewed towards the products (metabolites) compared to in conventional crops. Of the glyphosate tolerant crops, only GM varieties expressing the GAT enzyme introduces different types of metabolites in the crops compared to conventional crops. This GAT gene in glyphosate tolerant plants encodes an enzyme that effectively detoxifies glyphosate by *N*-acetylation (see chapter 5 for more details).

The primary degradation product of glyphosate in plants, soil and water is AMPA. To answer whether possible changes in the spectrum of metabolites of glyphosate may have implications for the toxicity of glyphosate tolerant crops (ToR 4), we have examined the chemical reactions performed by the GAT enzyme. GAT converts glyphosate to *N*-acetyl-glyphosate, which is further broken down to *N*-acetyl-aminomethyl phosphonic acid (*N*-acetyl-AMPA). Thus, the degradation of glyphosate in GAT-expressing plants results in four residues; glyphosate, *N*-acetyl-glyphosate, AMPA and *N*-acetyl-AMPA (EFSA, 2018b). The main question in this ToR is whether these three metabolites are more or less toxic than glyphosate for humans.

Previous relevant risk assessments of glyphosate and its metabolites in chronological order

The Joint FAO/WHO Meeting on Pesticide Residues (JMPR) is an expert *ad hoc* body administered jointly by FAO and WHO with the purpose of harmonizing the requirement and the risk assessment on the pesticide residues. The JMPR conducts scientific evaluations of pesticide residues in food. The output of JMPR constitutes the essential basis for Codex MRLs for food and agricultural commodities circulating in international trade, the health-based guidance for pesticides (i.e. ADIs and ARfDs) and recommends maximum residue levels to the governments of the member countries and regions. JMPR has evaluated the toxicity of glyphosate and its metabolites several times.

JMPR, 1986: JMPR evaluated glyphosate toxicologically and allocated an ADI of 0 - 0.3 mg/kg bw per day for the sum of glyphosate and the metabolite aminomethyl phosphonic acid (AMPA) (JMPR, 1986). Metabolic studies in plants showed that the metabolites were the same in glyphosate tolerant and susceptible crops but their relative distribution depended on the speed and extent of conversion to AMPA.

JMPR, 1997: The results of toxicological studies of the metabolite AMPA and of its parent compound glyphosate were compared based on new data (JMPR, 1997). AMPA was no more toxic than glyphosate (evaluated in 1994). In general, glyphosate and AMPA produced similar effects in experimental animals. Exceptions were the lesions in the salivary gland seen in the 90-day toxicity studies in mice and rats with glyphosate from the USA National Toxicology Program and the cataracts induced by glyphosate in a two-year combined toxicity and carcinogenicity study in rats. Such effects were not seen in other studies with glyphosate or AMPA. Estimate of ADI for humans was 0 - 0.3 mg/kg bw for the sum of glyphosate and AMPA.

JMPR, 2004: JMPR evaluated the toxicity of glyphosate and its metabolite AMPA (JMPR, 2004). An ADI of 0 – 1.0 mg/kg bw per day was established for glyphosate and AMPA based on a 2-year study in rats (salivary gland effects) and an uncertainty factor of 100. JMPR concluded that it was unnecessary to establish an acute reference dose (ARfD) for glyphosate.

JMPR, 2011: The Meeting concluded that the group ADI of 0 – 1 mg/kg bw per day established by the JMPR in 2004 for glyphosate and AMPA may also be applied to *N*-acetyl-glyphosate and *N*-acetyl-AMPA, as the available toxicological data showed that these plant metabolites have no greater toxicity than the parent glyphosate (JMPR, 2011). The JMPR decided in 2004 that an ARfD for glyphosate was unnecessary. The Meeting in 2011 concluded that it was not necessary to establish an ARfD for *N*-acetyl-glyphosate or *N*-acetyl-AMPA in view of their low acute toxicity and the absence of any toxicological effects that would be likely to be elicited by a single dose. An addendum to the toxicological monograph was prepared. Estimate of ADI for humans was therefore maintained as 0 – 1 mg/kg bw per day for the sum of glyphosate, *N*-acetyl-glyphosate, AMPA and *N*-acetyl-AMPA.

JMPR, 2016: The Meeting concluded that glyphosate is unlikely to be genotoxic at anticipated dietary exposures (JMPR, 2016). Several carcinogenicity studies in mice and rats were available. The Meeting concluded that glyphosate was not carcinogenic in rats but could not exclude the possibility that it was carcinogenic in mice at very high doses. In view of the absence of carcinogenic potential in rodents at human-relevant doses and the absence of genotoxicity by the oral route in mammals, and considering the epidemiological evidence from occupational exposures, the Meeting concluded that glyphosate was unlikely to pose a carcinogenic risk to humans from exposure through the diet. The Meeting reaffirmed the group ADI for the sum of glyphosate and its metabolites of 0 – 1 mg/kg bw per day on the basis of effects on the salivary gland in rats. The Meeting concluded that it was not necessary to establish an ARfD for glyphosate or its metabolites in view of its low acute toxicity.

EFSA, the main risk assessment body for chemicals in food and feed in the EU, has also evaluated the safety of glyphosate and its metabolites several times.

EFSA, 2009: The new metabolites from glyphosate found in genetically modified soybeans and maize containing the glyphosate-*N*-acetyltransferase (GAT) gene were evaluated by EFSA in 2009. The major metabolite in these soybean and maize varieties was *N*-acetyl-glyphosate. Parent glyphosate, *N*-acetyl-aminomethyl phosphonic acid (*N*-acetyl-AMPA) and aminomethyl phosphonic acid (AMPA) were found in low concentrations in the edible parts of the crops. The toxicological assessment of *N*-acetyl-glyphosate and *N*-acetyl-AMPA revealed that these metabolites were of no higher toxicological concern than the parent compound. They concluded that the ADI of 0.3 mg/kg bw per day established for glyphosate based on four long-term toxicity studies in rats may therefore also be applied to assess long-term consumer risk related to exposure to these substances (EFSA, 2009).

EFSA, 2015: Toxicological reference values of glyphosate and its metabolites were evaluated by EFSA in 2015. It was concluded that the metabolite of glyphosate aminomethylphosphonic acid (AMPA), had a similar toxicological profile as glyphosate. The ADI for both glyphosate and AMPA was established as 0.5 mg/kg bw per day, based on the maternal and developmental NOAEL of 50 mg/kg bw per day from several developmental toxicity studies in rabbits, and using a standard uncertainty factor of 100. The acute reference dose (ARfD) for glyphosate and AMPA was also found to be 0.5 mg/kg bw per day based on the same NOAEL from the same studies (EFSA, 2015).

EFSA, 2018: The toxicological profiles of the glyphosate metabolites *N*-acetyl-glyphosate and *N*-acetyl-AMPA were studied further by EFSA in 2018. It was concluded that the toxicological profile of glyphosate would also cover these two metabolites. Therefore, the same NOAEL and ADI and ARfD values as were used for glyphosate and AMPA were used also for these two acetylated metabolites (EFSA, 2018a).

For risk assessments, EFSA concluded that the sum of glyphosate, AMPA, *N*-acetyl-glyphosate and *N*-acetyl-AMPA, expressed as glyphosate, should be used (EFSA, 2018b).

Regarding evaluation of whether possible changes in the spectrum of metabolites of other relevant herbicides may have implications for the toxicity of glyphosate tolerant crops, two approaches were used. First, a literature search was performed, which returned no useful information to answer this question.

As described in chapter 5, the GAT enzyme is an *N*-acetyl transferase from *Bacillus licheniformis* that has been optimized by gene shuffling for acetylation of glyphosate (Siehl et al., 2007). The structure-activity data on this enzyme (Siehl et al., 2005; Siehl et al., 2007) indicated a narrow substrate range for native GAT and variants optimised for acetylation of glyphosate. According to the developers, appreciable activity required an amine-containing compound with a phosphonyl or phosphoryl group and a carboxyl group with a main chain length of five or fewer atoms. Of the numerous compounds that were tested, those that lied outside these parameters all failed to exhibit appreciable activity with native GAT or any variant (Siehl et al., 2007).

In the second approach, to answer whether possible changes in the spectrum of metabolites of other selective herbicides may have implications for the toxicity of glyphosate tolerant crops, expert judgement was used. As described in chapter 5, of the 15 herbicides, fungicides and insecticides listed in Table 3 that are likely to be used on GM maize or soybean with GAT activity, only dimethoat and acephate appear to have a chain length of five or fewer atoms, both have a carbonyl (modified carboxyl) group, an amino group and a P-containing group (Figure 20). It was considered by expert judgement that for the 15 specified chemicals such bindings would not occur or to a very limited degree. However, in order to ascertain if any of these compounds could be metabolised by GAT, further studies into the enzyme binding and kinetics of this reaction would be needed.

6.1 Summary

In ToR 4, an evaluation of whether possible changes in the spectrum of metabolites may have implications for the toxicity of glyphosate tolerant crops was performed. In GM crops that are tolerant to glyphosate due to modified versions of EPSPS, glyphosate metabolites are not expected to be different from those found in conventional crops. In crops with the GOX enzyme, the types of metabolites are also expected to be the same as in conventional crops, however, the balance between glyphosate and enzyme reaction products is more skewed towards the products (metabolites) compared to in conventional crops. These modifications of EPSPS and GOX most likely have no implications for the toxicity of glyphosate tolerant crops in terms of metabolites.

In GM crops that are tolerant to glyphosate due to GAT, three metabolites are formed: AMPA, *N*-acetyl-glyphosate and *N*-acetyl-AMPA. It was concluded in several risk assessments both by JMPR and EFSA that the toxicological profile of glyphosate would also cover these metabolites, and the health based guidance value (ADI) are the sum of glyphosate and the three metabolites. The changes in the spectrum of glyphosate metabolites in these GM crops are not likely to have implications for the toxicity of glyphosate tolerant crops.

Regarding evaluation of whether possible changes in the spectrum of metabolites of other relevant herbicides may have implications for the toxicity of glyphosate tolerant crops, the literature search returned no relevant articles. Based on structure-activity studies and expert judgement, it is unlikely that any of the 15 selected herbicides, fungicides and insecticides that may be used on GM maize or soybean with GAT activity would function as substrate for the GAT enzyme, and therefore no new metabolites of these pesticides are expected to occur. However, experimental data is needed to answer this part of the ToR with more certainty.

7 Uncertainties

ToR 1. A comparison between common weed control practices used with glyphosate tolerant crops and those used with conventional crops

- Regarding the conclusions in ToR 1, it is uncertain to which degree the available information on agricultural practices from other countries are relevant for Norway and/or Europe. Description of agricultural practices is mainly based on information from USA, South America and Australia. Countries in the northern hemisphere are different from countries in the southern hemisphere (South America and Australia) with regard to growing season and agricultural practices.
- Information on herbicide use in cultivation of GMHT crops and conventional crops are partly based on herbicide use statistics from the NASS-USDA Database. This database does, however, not differentiate between GM and conventional crops. Nonetheless, the changes in herbicide use since GM crops were introduced has been interpreted as an indication of differences in cultivation of GMHT and conventional crops.
- For the South American countries, Argentina and Brazil, the available data on pesticide use is even less detailed. The published data on total herbicides used per year are without specification of both active ingredients and crops, implying even higher uncertainties than in the data from USA.
- Data on oilseed rape is not included in the herbicide use statistics for USA nor in the databases used for the South American countries, contributing to uncertainty regarding agricultural practices and herbicide use on this crop.

ToR 2. Residue levels of glyphosate, its metabolites and other relevant herbicides used on glyphosate tolerant and conventional crops

- Data on residue levels of glyphosate, glyphosate metabolites and 'traditional' herbicides for GM and conventional maize, soybean and oilseed rape crops were not found in the open literature. Therefore, no conclusions on the differences in residue levels between GM and conventional crops could be drawn for ToR 2.

ToR 3. A description of how the genetic modification(s) used to make a plant tolerant against glyphosate may influence the metabolism of glyphosate or other plant protection products

and

ToR 4. An evaluation of whether possible changes in the spectrum of metabolites may have implications for the toxicity of glyphosate tolerant crops

- Sufficient data where not found in the open literature on whether the 15 herbicides, fungicides and insecticides that are likely to be used on GM maize, soybean or oilseed rape may be substrates for the GAT enzyme. Therefore, no firm conclusions on the influence on the metabolism of other plant protection products and the potential subsequent toxicity could be drawn. The 15 chosen pesticides investigated for ToR 3 and ToR 4 were the most used herbicides, fungicides and insecticides in maize and soybean crops. Other selection criteria for pesticides could have been chosen adding to the uncertainty of the conclusions for ToR 3 and ToR 4.

8 Conclusions with answers to the terms of reference

Available information was considered sufficient to answer ToR 1. However, data available from literature searches and monitoring programs were insufficient to answer the questions in ToR 2-4.

ToR 1. A comparison between common weed control practices used with glyphosate tolerant crops and those used with conventional crops

The effect of the increasing adoption of GMHT crops is reflected in the user statistics of herbicides in USA, where such crops are dominant. The most obvious trend in use of herbicides is the extensive increase in use of glyphosate. On a volume basis glyphosate contributed less than 5% of total herbicides used in maize and soybean cultivation in the early 1990's. In 2015, glyphosate constituted 76% of herbicides applied in soybeans and 37% in maize. Globally, the use of glyphosate increased almost 15-fold in 20 years, to reach 826 000 tons in 2014. In some countries in South America, there has been an approximately five-fold increase in the area load of herbicides after the introduction of GM crops. In Norway, no GM plants are approved for cultivation nor for import as food or feed.

In conventional crops, broad-spectrum herbicides such as glyphosate are mainly used for burndown of weeds after harvest or before planting. In glyphosate tolerant crops, glyphosate can also be applied to control weeds after emergence of the crops, which reduces the need for use of other more selective herbicides. The differences in patterns of herbicide use between conventional and GMHT crops may have effects on the level of residues of herbicides in the harvested crops.

The area loads of herbicides other than glyphosate used on soybean and maize in USA initially declined after introduction of glyphosate tolerant varieties. However, several studies have shown an increased use of non-glyphosate herbicides in glyphosate tolerant crops in recent years. This has been linked to the development of glyphosate resistant weeds, requiring farmers to include herbicides other than glyphosate in order to combat these weeds.

ToR 2. Residue levels of glyphosate, its metabolites and other relevant herbicides used on glyphosate tolerant and conventional crops

There was very little data available on glyphosate residue levels both in conventional and glyphosate tolerant maize, soybean and oilseed rape. The data collected from our literature searches were therefore insufficient for a systematic comparison of conventional and glyphosate tolerant maize, soybean and oilseed rape. Fourteen research papers were found to include glyphosate residues, some including metabolites. However, the data were heterogeneous and practically not comparable. Residues were measured in different parts of the plants (leaf, stem, seed etc.), application rates varied, as did seasonal treatment and sampling times. In addition, the available survey and monitoring data also did not distinguish between GM and conventionally grown crops regarding glyphosate residues. It was not possible to conclude whether these two types of crops differed regarding residue levels of glyphosate.

In accordance with ToR 2, the project sought to compare residue levels of 10 other relevant herbicides in maize and soybean crops, selected based on annual use in USA (in tons). However, the literature searches returned no relevant publications. In addition, the available survey and monitoring data also did not distinguish between GM and conventionally grown crops regarding the 10 herbicides. It was not possible to conclude whether these two types of crops differed regarding residue levels of the 10 selected herbicides.

ToR 3. A description of how the genetic modification(s) used to make a plant tolerant against glyphosate may influence the metabolism of glyphosate or other plant protection products

and

ToR 4. An evaluation of whether possible changes in the spectrum of metabolites may have implications for the toxicity of glyphosate tolerant crops

Literature searches performed to investigate whether or not genetic modifications used to make a plant tolerant against glyphosate may influence the metabolism of other plant protection products (PPPs), returned insufficient data to fully answer ToR 3. Out of the three main modifications used to make plants tolerant to glyphosate, only the introduction of a gene expressing the enzyme glyphosate *N*-acetyl transferase (GAT) results in new metabolites of glyphosate: *N*-acetyl-glyphosate and *N*-acetyl-AMPA. However, these metabolites were not considered likely to have implications for the toxicity of GAT-expressing maize, soybean and oilseed rape.

No relevant publications were found where either of the two enzymes glyphosate oxidoreductase (GOX) or GAT was tested for ability to metabolise any of 15 selected herbicides, fungicides and insecticides, selected based on annual use in USA (in tons). Three publications describing the GAT enzyme and relevant chemical group affinities, were identified. Based on structure-activity studies and expert judgement, it was considered unlikely that any of the 15 chosen pesticides would function as substrate for the GAT enzyme. Thus, it was unlikely that this enzyme would affect the metabolites and therefore the toxicity of these 15 pesticides. However, experimental data is needed to answer these ToRs with more certainty.

9 Data gaps

ToR 1. A comparison between common weed control practices used with glyphosate tolerant crops and those used with conventional crops

- Since no glyphosate tolerant GM crops are allowed to be grown in the EU or in Norway, information is limited from EU and lacking from Norway on agricultural practices for glyphosate tolerant maize, soybean and oilseed rape.
- Information on herbicide use in GMHT crops and conventional crops is partly based on herbicide use statistics database for USA. More than 90% of all soybean and maize grown in USA are GM. This database does, however, not differentiate between GM and conventional crops. Detailed information is therefore lacking on herbicides used separately with glyphosate tolerant crops and with conventional crops of maize and soybean.
- For Argentina and Brazil, the available data on pesticide use is even less detailed. The published data on total herbicides used per year are without specification of active ingredients or whether the crops are GM or not.
- Data on oilseed rape is not included in the herbicide use statistics for USA nor in the databases used for the South American countries.

ToR 2. Residue levels of glyphosate, its metabolites and other relevant herbicides used on glyphosate tolerant and conventional crops

- No information was found on residue levels of glyphosate, glyphosate metabolites and selective herbicides for GM versus conventional maize, soybean and oilseed rape.

ToR 3. A description of how the genetic modification(s) used to make a plant tolerant against glyphosate may influence the metabolism of glyphosate or other plant protection products

and

ToR 4. An evaluation of whether possible changes in the spectrum of metabolites may have implications for the toxicity of glyphosate tolerant crops

- No information was found on whether the 15 herbicides, fungicides and insecticides that are likely to be used on GM maize, soybean or oilseed rape may be substrates for the GAT enzyme.

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Appendix I

Search strategy in order to answer ToR 1

Search title: Agricultural practices in cultivation of glyphosate tolerant maize, soybean, and oilseed rape versus conventional counterparts

Contact: Kirsten Eline Rakkestad
Librarian: Nataliya Byelyey
Comments: Duplicate Check in EndNote
Articles before check: 1072, after: 1027 (total)

Data base: Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) 1946 to Present
Search date: March 9, 2018
Articles found: 555 (Medline)

1	exp ZEA MAYS/	27182
2	exp Soybeans/	19032
3	exp Brassica rapa/	1432
4	(maize or mays or soy* or rapeseed).tw.	79650
5	1 or 2 or 3 or 4	93343
6	Herbicides/	15374
7	PESTICIDES/	18023
8	(gl#phosate or gl#fosate or glyoxylate or Roundup or pesticide or herbicide or toleran* or resistan*).tw.	1078064
9	(GMO* or LMO* or GE or GM or transgen* or genetic*modif* or genetic*transform* or genetic*manipulat* or genetic*impruv* or genetic*engineer* or livingmodif*).tw.	203558
10	(reduc* adj7 (resistant weeds or nodulation or manganese uptake or nitrogen fixation or pollution*)).tw.	3198
11	7 or 8 or 9 or 10	1272022
12	Agriculture/	36206
13	(agricultur* adj3 (practic* or techniq* or mangement)).tw.	2780
14	(soil adj2 (system* or farm*)).tw.	2311

15	(cropping pattern or landuse).tw.	145
16	zero tillage.tw.	26
17	weed control.tw.	663
18	12 or 13 or 14 or 15 or 16 or 17	38648
19	5 and 11 and 18	574
20	limit 19 to yr="2008 -Current"	365

Data base:	Embase 1974 to 2018 march 09
Search date:	March 9, 2018
Articles found:	232

1	exp maize/	29629
2	exp soybean/	26774
3	exp rapeseed/	3605
4	(maize or mays or soy* or rapeseed).tw.	83646
5	1 or 2 or 3 or 4	90505
6	exp herbicide/	48960
7	pesticide/	33363
8	(gl#phosate or gl#fosate or glyoxylate or Roundup or pesticide or herbicide or toleran* or resistan*).tw.	1347709
9	(GMO* or LMO* or GE or GM or transgen* or genetic*modif* or genetic*transform* or genetic*manipulat* or genetic*impruv* or genetic*engineer* or livingmodif*).tw.	270061
10	(reduc* adj7 (resistant weeds or nodulation or manganese uptake or nitrogen fixation or pollution*)).tw.	4581
11	6 or 7 or 8 or 9 or 10	1641061
12	agriculture/	41545
13	(agricultur* adj3 (practic* or techniq* or mangement)).tw.	3288
14	(soil conservation adj2 (system* or farm*)).tw.	2915
15	(cropping pattern or landuse).tw.	241
16	zero tillage.tw.	40
17	weed control.tw.	786

18	12 or 13 or 14 or 15 or 16 or 17	44643
19	5 and 11 and 18	703
20	limit 19 to (embase and yr="2008 -Current")	147

Data base: Web of Science
Dato: March 9, 2018
Articles found: 900

# 7	**#5 AND #2 AND #1**	**506**
	Refined by: WEB OF SCIENCE CATEGORIES: (PLANT SCIENCES)	
	Indexes=SCI-EXPANDED, SSCI, A&HCI, ESCI Timespan=2008-2018	
# 6	#5 AND #2 AND #1	1.076
	Indexes=SCI-EXPANDED, SSCI, A&HCI, ESCI Timespan=2008-2018	
# 5	#4 OR #3	20,018
	Indexes=SCI-EXPANDED, SSCI, A&HCI, ESCI Timespan=2008-2018	
# 4	TOPIC: ("agricultural management practices") OR TOPIC: ("effective land use") OR TOPIC: ("soil conservation system") OR TOPIC: ("good agricultural practice") OR TOPIC: ("weed control practice")	552
# 3	TOPIC: (resistant weed) OR TOPIC: (manganese uptake) OR TOPIC: (nitrogen fixation) OR TOPIC: (nodulation) OR TOPIC: (conservation tillage) OR TOPIC: (zero tillage)	19,508
# 2	TOPIC: (glyphosate) OR TOPIC: (gliphosate) OR TOPIC: (glifasate) OR TOPIC: (glyfosate) OR TOPIC: (glyoxylate) OR TOPIC: (Roundup) OR TOPIC: (pecticide) OR TOPIC: (herbicide) OR TOPIC: (toleran*) OR TOPIC: (resistan*)	1,027,709
# 1	TOPIC: (maize) OR TOPIC: (mays) OR TOPIC: (soy*) OR TOPIC: (rapeseed)	108,233
	Indexes=SCI-EXPANDED, SSCI, A&HCI, ESCI Timespan=2008-2018	

Data base: AGRICOLA
Search date: March 9, 2018
Articles found: 54

Search = (maize or mays or soy? or rapeseed)[in Keyword Anywhere]AND(glyphosate or glyfosate or glifosate or glyoxylate)[in Keyword Anywhere]AND(agricultural management practices)[in Keyword Anywhere]OR(good agricultural practice)[in Keyword Anywhere]

Appendix II

Search strategy in order to answer ToR 2 i)

Search title: Levels of residues of glyphosate and its metabolites

Contact:	Kirsten Eline Rakkestad
Librarian:	Nataliya Byelyey
Comments:	Duplicate Check in EndNote Articles before check: 789, after: 546 (total)

Data base:	Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) 1946 to Present February 28, 2018
Search date:	February 28, 2018
Articles found:	353 (Medline)

1	exp Zea mays/ or exp Soybeans/ or exp Brassica rapa/	45799
2	(maize or mays or corn or soy$ or Glycine max or oilseedrape or Brassica napus ssp oleifera).tw.	99574
3	1 or 2	107015
4	(gl#phosate or gl#fosate or glyoxylate or metaboli* or AMPA or GlyGran or Glyfos or GlyphodinA or Glyphomax or GlyphosateCT or Herbatop or Hockey or Kickdown or Klinik or Lancer or Roundup Max or yerbimat or pondmaster or oxoacetic acidglyoxalate).tw.	1055963
5	((phosphonomethylglycine or phosphonomethylaminoacetic or phosphonomethylglycine or aminomethylphosphonic or carboxymethylaminomethylphosphonic or glyoxylic or aldehydoformic or alpha-ketoacetic or oxalaldehydic or aminomethanephosphonic) adj acid).tw.	1233
6	(oxalaldehydic acid 2-oxoethanoic acid or oxo-acetic acid glyoxalate).tw.	0
7	(N-acetylglyphosate or N-Acetyl-N-phosphonomethylglycine or glycine N-acetyl-N-phosphonomethyl or N-phosphonomethyl-N-acetylglycine or N-phosphonomethyl-N-acetyl glycine or 2-N-Phosphonomethylacetamidoacetic acid or N-acetyl-AMPA or aminomethyl phosphonic acid N-acetyl).tw.	3
8	4 or 5 or 6 or 7	1056855
9	((maximum residue or MRL) adj2 (level$ or limit$)).tw.	1805

10	(residue* or trace* or rest amount*).tw.	496829
11	9 or 10	496903
12	3 and 8 and 11	488
13	limit 12 to yr="1998 - 2018"	353

Data base:	Embase 1974 to 2018 February 28
Search date:	February 28, 2018
Articles found:	176

1	exp maize/	29561
2	exp soybean/	26714
3	Brassica rapa/	799
4	(maize or mays or soy* or Glycine max or rapeseed or Brassica napus).tw.	86221
5	1 or 2 or 3 or 4	99511
6	(gl#phosate or gl#fosate or N-phosphonomethylglycine or phosphonomethyliminoacetic acid or carboxymethylaminomethylphosphonic acid or GlyGran or Glyphodin or Glyphomax or Herbatop or Hockey or Kickdown or Klinik or Lancer or Roundup Max or yerbimat or Pondmaster).tw.	6080
7	(metaboli* or AMPA or aminomethylphosphonic acid).tw.	1294514
8	(glyoxylate or oxalaldehydic acid 2-oxoethanoic acid or oxo-acetic acid,glyoxalate, glyoxylic acid or aldehydoformic acid or alpha-ketoacetic acid).tw.	3000
9	6 or 7 or 8	1301510
10	exp waste/	174834
11	((maximum residue or MRL) adj3 (level$ or limit$)).tw.	2085
12	10 or 11	176335
13	5 and 9 and 12	176

Data base: Cochrane Database of Systematic Reviews
Search date: February 28, 2018
Articles found: 0

#1	MeSH descriptor: [Zea mays] explode all trees	180
#2	MeSH descriptor: [Soybeans] explode all trees	277
#3	MeSH descriptor: [Brassica rapa] explode all trees	6
#4	(maize or mays or corn or soy* or Glycine max or oilseedrape or Brassica napus ssp oleifera) .tw.	220
#5	#1 or #2 or #3 or #4	645
#6	(gl?phosate or gl?fosate or glyoxylate or metaboli* or AMPA or GlyGran or Glyfos or GlyphodinA or Glyphomax or GlyphosateCT or Herbatop or Hockey or Kickdown or Klinik or Lancer or Roundup Max or yerbimat or pondmaster or oxoacetic acidglyoxalate) .tw.	1970
#7	"maximum residue level":ti,ab,kw (Word variations have been searched)	0
#8	"maximum residue limit":ti,ab,kw (Word variations have been searched)	1
#9	"MRL":ti,ab,kw (Word variations have been searched)	17
#10	"residue":ti,ab,kw (Word variations have been searched)	871
#11	#7 or #8 or #9 or #10	886
#12	#5 and #6 and #11	0

Data base: Scorpus
Search date: February 28, 2018
Articles found: 230

(TITLE-ABS-KEY (maize OR mays OR corn OR soy* OR "Glycine max" OR "oilseed rape" OR "Brassica napus ssp oleifera") AND TITLE-ABS-KEY (glyphosate OR glyfosate OR glyoxylate OR gliphosate OR glifosate OR metabolit*) AND TITLE-ABS-KEY ("maximum residue level" OR "maximum residue limit" OR mrl) OR TITLE-ABS-KEY (residue* OR trace* OR "rest amount*")) AND (LIMIT-TO (SUBJAREA , "AGRI")) AND (LIMIT-TO (PUBYEAR , 2018) OR LIMIT-TO (PUBYEAR , 2017) OR LIMIT-TO (PUBYEAR , 2016) OR LIMIT-TO (PUBYEAR , 2015) OR LIMIT-TO (PUBYEAR , 2014) OR LIMIT-TO (PUBYEAR , 2013) OR LIMIT-TO (PUBYEAR , 2012) OR LIMIT-TO (PUBYEAR , 2011) OR LIMIT-TO (PUBYEAR , 2010) OR LIMIT-TO (PUBYEAR , 2009) OR LIMIT-TO (PUBYEAR , 2008) OR LIMIT-TO (PUBYEAR , 2007) OR LIMIT-TO (PUBYEAR , 2006) OR LIMIT-TO (PUBYEAR , 2005) OR LIMIT-TO (PUBYEAR , 2004) OR LIMIT-TO (PUBYEAR , 2003) OR LIMIT-TO (PUBYEAR , 2002) OR LIMIT-TO (PUBYEAR , 2001) OR LIMIT-TO (PUBYEAR , 2000) OR LIMIT-TO (PUBYEAR , 1999) OR LIMIT-TO (PUBYEAR , 1998))

Database: Web of Science
Search date: February 28, 2018
Articles found: 20

1	TOPIC: (maize) OR TOPIC: (mays) OR TOPIC: (corn) OR TOPIC: (soy*) OR TOPIC: (oilseedrape)	254489
2	TOPIC: (glyphosate) OR TOPIC: (glyfosate) OR TOPIC: (gliphosate) OR TOPIC: (glifosate) OR TOPIC: (glifosate) OR TOPIC: (matabol*) OR TOPIC: (AMPA)	26451
3	TOPIC: ("maximum residue level") OR TOPIC: ("maximum residue limit")	1164
4	#1 AND #2 AND #3 Refined by: PUBLICATION YEARS: (2012 OR 2010 OR 2008 OR 2017 OR 2016 OR 2011)	18

Data base: JSTOR
Search date: February 28, 2018
Articles found: 12

(((maize OR corn OR soya OR oilseed rape) AND (glyphosate OR glyfosate OR gliphosate OR glifosate OR glyoxylate)) AND (maximum residue level))

Appendix III

Search strategy in order to answer ToR2 ii)

Search title: Residues of 10 selected herbicides in maize and soybean

Contact:	Ville Erling Sipinen
Librarian:	Nataliya Byelyey
Comment:	Duplicate check in EndNote Articles before check: 200, after: 134 (total)
Data base:	Epub Ahead of Print, In-Process & Other Non- Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) 1946 to Present
Search date:	May 22, 2018
Articles found:	21 (Medline)

1	exp maize/ or exp soybean/	44989
2	(maize or mays or soy*).tw.	78279
3	1 or 2	91098
4	exp atrazine/	2574
5	gesamprim.tw.	1
6	6-chloro-N2-ethyl-N4-propan-2-yl-1,3,5-triazine-2,4-diamine.tw.	0
7	1-Chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine.tw.	6
8	6-Chloro-N-ethyl-N'-1-methylethyl-1,3,5-triazine-2,4-diamine.tw.	18
9	a?etochlor.tw.	318
10	2-Chloro-N-ethoxymethyl-N-2-ethyl-6-methylphenylacetamide.tw.	0
11	(metolachlor or dual or pimagram or bicep or pennant).tw.	172979
12	CGA-24705.tw.	0
13	RS-2-Chloro-N-2-ethyl-6-methyl-phenyl-N-1-methoxypropan-2-ylacetamide.tw.	0
14	(dimethenamid or "frontier herbicide").tw.	32
15	San682H.tw.	0
16	RS-2-Chloro-N-2,4-dimethyl-3-thienyl-N-2-methoxy-1-methylethylacetamide.tw.	0
17	exp simazine/	351
18	(gesatop pr princep or herbazin or simanex or aquazine).tw.	5

19	6-Chloro-N,N'-diethyl-1,3,5-triazine-2,4-diamine.tw.	7
20	(clopyralid? or lontrel or benzalox).tw.	65
21	3,6-Dichloropicolinic acid.tw.	12
22	3,6-Dichloropyridine-2-carboxylic acid.tw.	3
23	(fomesafen? or Reflex).tw.	66412
24	UNII-M0A3U4CDTF.tw.	0
25	HSDB 6660.tw.	0
26	PP021.tw.	2
27	5-2-Chloro-4-trifluoromethylphenoxy-N-methylsulfonyl-2-nitrobenzamide.tw.	0
28	sulfentrazone.tw.	31
29	F6285.tw.	0
30	FP846.tw.	1
31	N-2,4-dichloro-5-4-difluoromethyl-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-ylphenylmethanesulfonamide.tw.	0
32	Authority.tw.	19084
33	(metribuzin or lexone or zenkor or sencorex).tw.	249
34	"Bay94337".tw.	0
35	(pendimethalin or prowl or penoxaline or herdabox).tw.	297
36	exp paraquat/	4895
37	(paraquat or gramaxone or "methyl viologen" or "paragreen A" or pathclear or pillarxone).tw.	7236
38	1-methyl-4-1-methylpyridin-1-ium-4-ylpyridin-1-iumdichloride.tw.	0
39	4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38	269249
40	(residue? adj3 (level$ or concentration)).tw.	3639
41	(trace? or rest amount?).tw.	146341
42	40 or 41	149843
43	3 and 39 and 42	21

Data base: Embase 1974 to 2017 May 25
Search date: May 22, 2018
Articles found: 32

1	exp maize/ or exp soybean/	54842
2	(maize or mays or soy*).tw.	81995
3	1 or 2	95279
4	exp atrazine/	5891
5	gesamprim.tw.	1
6	6-chloro-N2-ethyl-N4-propan-2-yl-1,3,5-triazine-2,4-diamine.tw.	0
7	1-Chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine.tw.	6
8	6-Chloro-N-ethyl-N'-1-methylethyl-1,3,5-triazine-2,4-diamine.tw.	48
9	exp acetochlor/	400
10	a?etochlor.tw.	340
11	2-Chloro-N-ethoxymethyl-N-2-ethyl-6-methylphenylacetamide.tw.	0
12	(metolachlor or dual or pimagram or bicep or pennant).tw.	219320
13	CGA-24705.tw.	0
14	RS-2-Chloro-N-2-ethyl-6-methyl-phenyl-N-1-methoxypropan-2-ylacetamide.tw.	0
15	(dimethenamid or "frontier herbicide").tw.	34
16	San682H.tw.	0
17	RS-2-Chloro-N-2,4-dimethyl-3-thienyl-N-2-methoxy-1-methylethylacetamide.tw.	0
18	exp simazine/	1464
19	(gesatop pr princep or herbazin or simanex or aquazine).tw.	7
20	6-Chloro-N,N'-diethyl-1,3,5-triazine-2,4-diamine.tw.	12
21	(clopyralid? or lontrel or benzalox).tw.	80
22	3,6-Dichloropicolinic acid.tw.	17
23	3,6-Dichloropyridine-2-carboxylic acid.tw.	5
24	exp fomesafen/	71
25	(fomesafen? or Reflex).tw.	79803
26	UNII-M0A3U4CDTF.tw.	0

27	HSDB 6660.tw.	0
28	PP021.tw.	2
29	5-2-Chloro-4-trifluoromethylphenoxy-N-methylsulfonyl-2-nitrobenzamide.tw.	0
30	sulfentrazone.tw.	30
31	F6285.tw.	0
32	FP846.tw.	1
33	N-2,4-dichloro-5-4-difluoromethyl-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-ylphenylmethanesulfonamide.tw.	0
34	Authority.tw.	24977
35	exp metribuzin/	454
36	(metribuzin or lexone or zenkor or sencorex).tw.	354
37	"Bay94337".tw.	0
38	exp pendimethalin/	411
39	(pendimethalin or prowl or penoxaline or herdabox).tw.	363
40	exp paraquat/	8092
41	(paraquat or gramaxone or "methyl viologen" or "paragreen A" or pathclear or pillarxone).tw.	8147
42	1-methyl-4-1-methylpyridin-1-ium-4-ylpyridin-1-iumdichloride.tw.	0
43	4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42	340116
44	exp residue analysis/	7765
45	(residu* adj3 (level$ or concentration)).tw.	10497
46	(trace* or rest amount*).tw.	202611
47	44 or 45 or 46	219755
48	3 and 43 and 47	46
49	limit 48 to embase	32

Data base: Web of Science
Search date: May 22, 2018
Articles found: 147

# 5	92	#3 AND #2 AND #1
		Refined by: WEB OF SCIENCE CATEGORIES: (PLANT SCIENCES)
		Indexes=SCI-EXPANDED, SSCI Timespan=1987-2018
# 4	435	#3 AND #2 AND #1
		Indexes=SCI-EXPANDED, SSCI Timespan=1987-2018
# 3	3,282,989	TOPIC: ("resedue? level") OR TOPIC: (concentration) OR TOPIC: (trace?) OR TOPIC: (amount*)
		Indexes=SCI-EXPANDED, SSCI Timespan=1987-2018
# 2	22,039	TOPIC: (atrazine) OR TOPIC: (acetochlor) OR TOPIC: (metolachlor) OR TOPIC: (dimethenamid) OR TOPIC: (simazine) OR TOPIC: (clopyralid) OR TOPIC: (fomezafen) OR TOPIC: (sulfentrazone) OR TOPIC: (metribuzin) OR TOPIC: (pendimethalin) OR TOPIC: (paraquat)
		Indexes=SCI-EXPANDED, SSCI Timespan=1987-201
# 1	202,983	TOPIC: (maize) OR TOPIC: (mays) OR TOPIC: (soy*)
		Indexes=SCI-EXPANDED, SSCI Timespan=1987-2018

Appendix IV

Search strategy in order to answer ToR 3 and ToR4.

Search title: Plant protection products (PPPs) and metabolism by glyphosate *N*-acetyl transferase (GAT) / glyphosate oxidoreductase (GOX) GOXv247

Contact: Ville Erling Sipinen
Librarian: Nataliya Byelyey
Comment: Duplicate check in EndNote
Articles before check: not applicable (NA), after: NA

Data base: Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) 1946 to Present
Search date: May 25, 2018
Articles found: 0

1	exp ATRAZINE/	2574
2	(Atrazine or Gesamprim).tw.	4034
3	6-chloro-N2-ethyl-N4-propan-2-yl-1,3,5-triazine-2,4-diamine.tw.	0
4	1-Chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine.tw.	6
5	6-Chloro-N-ethyl-N'-1-methylethyl-1,3,5-triazine-2,4-diamine.tw.	18
6	1 or 2 or 3 or 4 or 5	4215
7	(acetochlor or azetochlor).tw.	318
8	2-Chloro-N-ethoxymethyl-N-2-ethyl-6-methylphenylacetamide.tw.	0
9	(metolachlor or dual or pimagram or bicep or pennant).tw.	172979
10	RS-2-Chloro-N-2-ethyl-6-methyl-phenyl-N-1-methoxypropan-2-ylacetamide.tw.	0
11	mesotrione.tw.	130
12	2-4-Methylsulfonyl-2-nitrobenzoylcyclohexane-1,3-dione.tw.	0
13	exp 2,4-Dichlorophenoxyacetic Acid/	2703
14	2,4-D.tw.	3439
15	2,4 dichlorophenoxyacetic acid.tw.	2379
16	(hedonal or trinoxol).tw.	9
17	7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16	178462

18	exp DICAMBA/	163
19	(dicamba or dianat).tw.	316
20	3,6-Dichloro-o-anisic acid.tw.	10
21	"3,6-Dichloro-2-methoxybenzoic acid".tw.	20
22	18 or 19 or 20 or 21	344
23	(pyraclostrobin* or pyrachlostrobin*).tw.	192
24	methyl 2-1-4-chlorophenylpyrazol-3-yloxymethyl-N-methoxycarbanilate.tw.	0
25	methyl N-2-1-4-chlorophenyl-1H-pyrazol-3-yloxymethylphenyl-N-methoxycarbamate.tw.	0
26	propiconazole.tw.	336
27	1-2-2,4-dichlorophenyl-4-propyl-1,3-dioxolan-2-ylmethyl-1,2,4-triazole.tw.	0
28	23 or 24 or 26 or 27	516
29	(azoxystrobin or azoxystrobine or heritage or amistar or quadris or bankit).tw.	5520
30	methyl2E-2-2-6-2-cyanophenoxypyrimidin-4-yloxyphenyl-3-methoxyacrylate.tw.	0
31	exp CHLORPYRIFOS/	2649
32	(chlorpyrifos* or dursban or lorsban or brodan or bolton or cobalt or "detmol UA" or "Dowco 179" or empire or oeradex or hatchet or nufos or paqeant pr piridane or scout or stipend or tricel or warhawk).tw.	38318
33	Bifenthrin*.tw.	701
34	2-Methyl-3-phenylphenylmethyl1S,3S-3-Z-2-chloro-3,3,3-trifluoroprop-1-enyl-2,2-dimethylcyclopropane-1-carboxylate.tw.	0
35	29 or 30 or 31 or 32 or 33 or 34	44633
36	(propargite or omite or comite or "Uniroyal D014").tw.	454
37	2-4-tert-butylphenoxycyclohexyl prop-2-yne-1-sulfonate.tw.	0
38	cyhalotrin*.tw.	3
39	3-2-chloro-3,3,3-trifluoro-1-propenyl-2,2-dimethyl-cyano3-phenoxyphenylmethylcyclopropanecarboxylate.mp.	0
40	dimetoat*.tw.	9
41	O,O-dimethyl S-methylcarbamoylmethyl phosphorodithioate.tw.	3
42	Phosphorodithioic acid,O,O-Dimethyl S-2-methylamino-2-oxoethylylester.tw.	0

43	O,O-dimethyl S-2-methylamino-2-oxoethyldithiophosphate.tw.	0
44	acephate.tw.	377
45	N-Methoxy-methylsulfanylphosphorylacetamide.tw.	0
46	36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44 or 45	846
47	6 or 17 or 22 or 28 or 35 or 46	227399
48	Glyphosate N-acetyl transferase.tw.	0
49	("Glyphosate oxidoreductase" or "Glyphosate acetyltransferase").tw.	19
50	48 or 49	19
51	46 and 50	0

Data base: Embase 1974 to 2018 May 21
Search date: May 25, 2018
Articles found: 0

1	exp atrazine/	5891
2	(Atrazine or Gesamprim).tw.	5158
3	6-chloro-N2-ethyl-N4-propan-2-yl-1,3,5-triazine-2,4-diamine.tw.	0
4	1-Chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine.tw.	6
5	6-Chloro-N-ethyl-N'-1-methylethyl-1,3,5-triazine-2,4-diamine.tw.	48
6	1 or 2 or 3 or 4 or 5	6553
7	exp acetochlor/	400
8	(acetochlor or azetochlor).tw.	340
9	2-Chloro-N-ethoxymethyl-N-2-ethyl-6-methylphenylacetamide.tw.	0
10	7 or 8 or 9	475
11	exp metolachlor/	1083
12	(metolachlor or dual or pimagram or bicep or pennant).tw.	219320
13	RS-2-Chloro-N-2-ethyl-6-methyl-phenyl-N-1-methoxypropan-2-ylacetamide.tw.	0
14	11 or 12 or 13	219594
15	mesotrione.tw.	134
16	2-4-Methylsulfonyl-2-nitrobenzoylcyclohexane-1,3-dione.tw.	0
17	15 or 16	134

18	exp 2,4 dichlorophenoxyacetic acid/	4338
19	2,4-D.tw.	4158
20	2,4 dichlorophenoxyacetic acid.tw.	2286
21	(hedonal or trinoxol).tw.	5
22	18 or 19 or 20 or 21	6164
23	exp dicamba/	438
24	3,6-Dichloro-o-anisic acid.tw.	17
25	"3,6-Dichloro-2-methoxybenzoic acid".tw.	41
26	23 or 24 or 25	454
27	(pyraclostrobin* or pyrachlostrobin*).tw.	188
28	methyl 2-1-4-chlorophenylpyrazol-3-yloxymethyl-N-methoxycarbanilate.tw.	0
29	methyl N-2-1-4-chlorophenyl-1H-pyrazol-3-yloxymethylphenyl-N-methoxycarbamate.tw.	0
30	27 or 28 or 29	188
31	exp propiconazole/	547
32	1-2-2,4-dichlorophenyl-4-propyl-1,3-dioxolan-2-ylmethyl-1,2,4-triazole.tw.	0
33	propiconazole.tw.	402
34	31 or 32 or 33	609
35	(azoxystrobin or azoxystrobine or heritage or amistar or quadris or bankit).tw.	6093
36	methyl2E-2-2-6-2-cyanophenoxypyrimidin-4-yloxyphenyl-3-methoxyacrylate.tw.	0
37	35 or 36	6093
38	exp chlorpyrifos/	5628
39	(chlorpyrifos* or dursban or lorsban or brodan or bolton or cobalt or "detmol UA" or "Dowco 179" or empire or oeradex or hatchet or nufos or paqeant pr piridane or scout or stipend or tricel or warhawk).tw.	40475
40	38 or 39	42058
41	exp bifenthrin/	655
42	2-Methyl-3-phenylphenylmethyl1S,3S-3-Z-2-chloro-3,3,3-trifluoroprop-1-enyl-2,2-dimethylcyclopropane-1-carboxylate.tw.	0
43	bifenthrin*.tw.	704

44	41 or 42 or 43	894
45	(propargite or omite or comite or "Uniroyal D014").tw.	458
46	2-4-tert-butylphenoxycyclohexyl prop-2-yne-1-sulfonate.tw.	0
47	45 or 46	458
48	exp cyhalothrin/	1241
49	cyhalotrin*.tw.	5
50	3-2-chloro-3,3,3-trifluoro-1-propenyl-2,2-dimethyl-cyano3-phenoxyphenylmethylcyclopropanecarboxylate.tw.	0
51	48 or 49 or 50	1242
52	dimetoat*.tw.	11
53	O,O-dimethyl S-methylcarbamoylmethyl phosphorodithioate.tw.	3
54	Phosphorodithioic acid,O,O-Dimethyl S-2-methylamino-2-oxoethylylester.tw.	0
55	O,O-dimethyl S-2-methylamino-2-oxoethyldithiophosphate.tw.	0
56	52 or 53 or 54 or 55	14
57	exp acephate/	538
58	acephate.tw.	447
59	N-Methoxy-methylsulfanylphosphorylacetamide.tw.	0
60	57 or 58 or 59	653
61	6 or 10 or 14 or 17 or 22 or 26 or 30 or 34 or 37 or 40 or 44 or 47 or 51 or 56 or 60	281690
62	Glyphosate N-acetyl transferase.tw.	0
63	("Glyphosate oxidoreductase" or "Glyphosate acetyltransferase").tw.	19
64	62 or 63	19
65	61 and 64	0

Data base: Web of Science
Search date: May 25, 2018
Articles found: 0

#20	#19 AND #18 AND #15 *DocType=All document types; Language=All languages;*	0
#19	TOPIC: (metabolism) OR TOPIC: (acetylation) OR TOPIC: ("N-acetylation")	646,709

	Indexes=SCI-EXPANDED, SSCI Timespan=1987-2018	
#18	#17 OR #16 *DocType=All document types; Language=All languages;*	54
#17	TOPIC: (glyphosate oxidoreductase) *OR* TOPIC: (glyphosate acetyltransferase) *DocType=All document types; Language=All languages;*	54
#16	TOPIC: (glyphosate N-acetyl transferase) *DocType=All document types; Language=All languages;*	2
#15	#14 OR #13 OR #12 OR #11 OR #10 OR #9 OR #8 OR #7 OR #6 OR #5 OR #4 OR #3 OR #2 OR #1 *DocType=All document types; Language=All languages;*	541,862
#14	TOPIC: (acephate) *OR* TOPIC: (N-(Methoxy-methylsulfanylphosphoryl)acetamide) *DocType=All document types; Language=All languages;*	714
#13	TOPIC: (dimetoat) *OR* TOPIC: (dimethoate) *OR* TOPIC: (O,O-dimethyl S-methylcarbamoylmethyl phosphorodithioate) *OR* TOPIC: (Phosphorodithioic acid, O,O-Dimethyl S-(2-(methylamino)-2-oxoethylyl)ester) *OR* TOPIC: (o O,O-dimethyl S-[2-(methylamino)-2-oxoethyl] dithiophosphate) *DocType=All document types; Language=All languages;*	1,635
#12	TOPIC: (λ-cyhalotrin) *OR* TOPIC: (cyhalothrine) *OR* TOPIC: (3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethyl-cyano(3- phenoxyphenyl)methyl cyclopropanecarboxylate) *DocType=All document types; Language=All languages;*	2
#11	TOPIC: (propargite) *OR* TOPIC: (omite) *OR* TOPIC: (comite) *OR* TOPIC: ("uni royal D014") *OR* TOPIC: (2-(4-tert-butylphenoxy)cyclohexyl prop-2-yne-1-sulfonate) *DocType=All document types; Language=All languages;*	732
#10	TOPIC: (bifenthrin*) *DocType=All document types; Language=All languages;*	1,161
#9	TOPIC: (chlorpyrifos) *OR* TOPIC: (dursban) *OR* TOPIC: (lorsban) *OR* TOPIC: (brodan) *OR* TOPIC: (bolton) *OR* TOPIC: (chlorpyrifos-ethyl) *OR* TOPIC: (cobalt) *OR* TOPIC: ("Detmol UA") *OR* TOPIC: ("Dowco 179") *OR* TOPIC: (empire) *OR* TOPIC: (eradex) *OR* TOPIC: (hatchet) *OR* TOPIC: (nufos) *OR* TOPIC: (paqeant) *OR* TOPIC: (piridane) *OR* TOPIC: (scout)*OR* TOPIC: (stipend) *OR* TOPIC: (tricel) *OR* TOPIC: (warhawk) *OR* TOPIC: (O, O-Diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate) *DocType=All document types; Language=All languages;*	168,952
#8	TOPIC: (azoxystrobin*) *OR* TOPIC: (heritage) *OR* TOPIC: (amistar) *OR* TOPIC: (quadris) *OR* TOPIC: (bankit) *OR* TOPIC: (methyl (2E)-2-(2-{[6-(2-	37,6584

	cyanophenoxy)pyrimidin-4-yl]oxy}phenyl)-3-methoxyacrylate) *DocType=All document types; Language=All languages;*	
#7	TOPIC: (Pyraclostrobin) *OR* TOPIC: (Pyrachlostrobin*) *OR* TOPIC: (methyl 2-(1-(4-chlorophenyl)pyrazol-3-yloxymethyl)-N-methoxycarbanilate) *OR* TOPIC: (methyl N-(2-(1-(4-chlorophenyl)-1H-pyrazol-3-yloxymethyl)phenyl)-(N-methoxy)carbamate) *DocType=All document types; Language=All languages;*	650
#6	TOPIC: (dicamba) *OR* TOPIC: (dianat) *OR* TOPIC: (3,6-Dichloro-o-anisic acid) *OR* TOPIC: (3,6-Dichloro-2-methoxybenzoic acid) *DocType=All document types; Language=All languages;*	1,252
#5	TOPIC: (2,4-D) *OR* TOPIC: (2,4 dichlorophenoxyacetic acid) *OR* TOPIC: (hedonal) *OR* TOPIC: (dianat) *OR* TOPIC: (3,6-Dichloro-o-anisic acid) *OR* TOPIC: (3,6-Dichloro-2-methoxybenzoic acid) *DocType=All document types; Language=All languages;*	10,701
#4	TOPIC: (mesotrione) *OR* TOPIC: (2-[4-(Methylsulfonyl)-2-nitrobenzoyl]cyclohexane-1,3-dione) *DocType=All document types; Language=All languages;*	434
#3	TOPIC: (metolachlor) *OR* TOPIC: (dual) *OR* TOPIC: (pimagram) *OR* TOPIC: (bicep) *OR* TOPIC: (pennant) *OR* TOPIC: ((RS)-2-Chloro-N-(2-ethyl-6-methyl-phenyl)-N-(1-methoxypropan-2-yl)acetamide) *DocType=All document types; Language=All languages;*	343,293
#2	TOPIC: (acetochlor) *OR* TOPIC: (azetochlor) *OR* TOPIC: (2-Chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)acetamide) *DocType=All document types; Language=All languages;*	662
#1	TOPIC: (atrazine) *OR* TOPIC: (gesamprim) *OR* TOPIC: (6-chloro-N2-ethyl-N4-(propan-2-yl)-1,3,5-triazine-2,4-diamine) *OR* TOPIC: (1-Chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine) *DocType=All document types; Language=All languages;*	10,755