The Schumacher Institute

Novel Genetic Entities

GMOs within the Planetary Boundaries Framework

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1 Introduction

The planetary boundaries framework was developed by the Stockholm Resilience Centre and first published in 2009, with an update published in 2015 (1, 2). The aim was to define a 'safe operating space' of human activity concerning the functioning of the Earth System. They set out nine key processes, the transgression of which would result in unacceptable levels of global change, and quantified boundaries for seven on the nine key processes. The boundary sets out the safe operating space (safe), up to a zone of uncertainty (increasing risk), wherein the effects of the boundary transgression become unpredictable. Beyond the zone of uncertainty, is the 'dangerous level' of boundary transgression (high risk), at which point boundary transgression reaches unacceptably high levels with dangerous and difficult to reverse effects. A summary of the nine planetary boundaries can be found in table 1.

Earth System Process	Boundary	Overall Quantification as of 2015 (2)
Climate Change	Atmospheric CO_2 concentration, ppm = 350ppm CO_2 Energy imbalance at top of atmosphere W m^{-2} = +1.0 W m^{-2}	Increasing Risk
Biosphere Integrity (previously Biodiversity Loss)	Genetic Diversity: Extinction rate = <10E/MSY Functional Diversity: Biodiversity Intactness Index (BII) = >90%	Genetic Diversity: High Risk Functional Diversity: Unquantified
Land-System Change (previously Land Use Change)	Area of forested land as % of original forest cover = 75% Tropical: 85%, Temperate: 50%, Boreal: 85%	Increasing Risk
Freshwater Use	Maximum amount of consumptive blue water use (km^3yr^{-1}) = 4000km^3yr^{-1}	Safe

Biochemical Flows of Phosphorus and Nitrogen	P: Flow from freshwater systems into ocean = 11 Tg P yr^{-1}, flow from fertilisers to erodible soils = 6.2 Tg yr^{-1} N: Industrial and intentional biological fixation of N = 62 Tg N yr^{-1}	Phosphorus: High Risk Nitrogen: High Risk
Ocean Acidification	Carbonate ion concentration = >80% of pre-industrial saturation	Safe
Atmospheric Aerosol Loading	Unquantified	
Stratospheric Ozone Depletion	Stratospheric O_3 concentration, DU = <5% reduction from preindustrial level of 290 DU	Safe
Novel Entities (previously Chemical Pollution)	Unquantified	

Table 1: Summary of nine key Earth System processes of which seven have some planetary boundary quantification, description of quantification and planetary boundary status, as of 2015 (1, 2).

Of the planetary boundaries, seven have some form of quantification. Atmospheric aerosol loading has a quantified example at a local level, however, novel entities remain unquantified. In the following section, the current understanding of 'novel entities' is discussed.

1.1 Novel Entities

Novel entities were originally categorised in the planetary boundaries framework in 2009 as 'chemical pollution', consisting of compounds and substances with effects on ecosystem and Earth System functioning, such as persistent organic pollutants (POPs), plastics, endocrine disruptors, heavy metals and nuclear wastes (1). The category was expanded as 'novel entities', defined as "new substances, new forms of existing substances, and modified life forms that have the potential for unwanted geophysical and/or biological effects" that are "of concern at the global level when these entities exhibit (i) persistence, (ii) mobility across scales with consequent widespread distributions, and (iii) potential impacts on vital Earth-System processes or subsystems" (2). This broadened category included the substances previously identified as chemical pollutants, but also identified the potential for the huge variety of substances, materials and life forms which could affect ecosystem and Earth System functioning. The 'chemical

pollution/novel entities' category has been the subject of some discussion, in examining what constitutes a 'novel entity' and what might be included in this planetary boundary category (3-7). A list of potential novel entities and examples of such novel entities are described in table 2.

Novel Entity	Description
Toxic Compounds and Substances: Chemical Pollutants	Groups of chemicals, radioactive materials and/or nanomaterials exhibiting persistence and undergo long-range transport, and have adverse interactions with organisms or otherwise disrupt ecosystem services and/or key Earth System processes, e.g. chlorofluorocarbons (CFCs), persistent organic pollutants (POPs), endocrine disruptors.
Toxic Compounds and Substances: Radioactive Materials	
Toxic Compounds and Substances: Nanomaterials	
Toxic Compounds and Substances: Technology-Critical Elements	Rare earth elements, platinum group elements, other scarce materials. Essential for manufacture of current and emerging technologies. Potential toxicity for plants, animals and other organisms, or for ecological disruption.
Plastics and Micro-Plastics	Synthetic polymer-based plastic waste, and small plastic particulates <5 mm in size released into the environment, in particular river and ocean pollution. Sources include industrial waste, tyres, textiles, cosmetics and cleaning products.
Genetically Modified Organisms	Any organism in which the genetic material has been altered in a way that would not occur by mating and/or natural recombination.
Cellular Agriculture	Production of animal/livestock products with minimal/no use for live animals, e.g. for food, leather, fur.
Engineered Bio-Based Materials and Compounds	Use of synthetic biology to produce materials such as biofuels, plastics and chemicals.

Table 2: Categories of potential novel entities with examples of novel entities in each category. Discussed in (1, 2, 4, 6, 8-10).

The 'novel entities' category is extremely broad; estimates for the number of chemicals in production since the 1950 range from 100,000-140,000 (1, 2, 7). Furthermore, an approach of assessing toxicity and ecotoxicity of single chemicals may not be sufficient, as mixtures of chemicals may have toxicity disproportionate to that of each chemical individually (6, 7). Adverse effects of chemical or other novel entities do not necessarily have linear dose-response relationships; strong adverse effects can be observed even at very low concentrations and increase or decrease with concentration.

Novel entities can disrupt Earth System processes by aggregating to a level that causes adverse effects on human health, reduces the resilience of ecosystems, and undermines the resilience of ecosystems such that they are more susceptible to transgressions of other planetary boundaries e.g., changes in land use, biodiversity loss (1, 7). Novel entities can directly influence other aspects of the planetary boundaries, for example, the reduction of biodiversity resulting from the widespread use of biocides. Persson *et al* suggested three conditions for a chemical compound, substance, or lifeform to be considered a novel entity (3):

1. The chemical or mixture of chemicals has a disruptive effect on a vital Earth System process.
2. The disruptive effect is not discovered until it is, or inevitably will become, a problem at a planetary scale.
3. The effects of the pollutant in the environment cannot readily be reversed.

Such an example would be a broad range of herbicide. A broad range of herbicide has the potential when in widespread heavy use, to disrupt biodiversity by damaging or killing large numbers of target and non-target plants. Through widespread and persistent use, the removal of large swathes of plant biodiversity can become an extensive problem, severely disrupting ecosystem functioning by changes to interactions with other organisms and ecosystem services. Even after the effects of the herbicide are discovered, such large-scale changes to ecosystem functioning are difficult to reverse; even if possible, ecosystem recovery can take years, decades, or centuries, dependent on the extent of the damage. In such a case, the hypothetical herbicide is acting as a novel entity.

In the example, an essential aspect of the novel entity acting as a planetary boundary threat is the component of human activity. The example herbicide would create more damage to ecosystem processes if it were applied at high concentrations over a large area, and if applied non-selectively. At lower concentrations, over a small area, and with a targeted application, such a herbicide is still a novel entity, but unlikely to breach a planetary boundary. An even starker example of the role of human activity in threats to a novel entity planetary boundary is that of the technology critical elements, which only become novel entities after mining and through the processes of refinement and subsequent use; before extraction, such elements pose little if any novel entity threat.

The broadness of the 'novel entities' category makes defining a planetary boundary complex. To define a planetary boundary, the ultimate effect or response variable and the critical control variable must first be defined. In the case of novel entities, the response variable is the adverse ecological/human health impact caused by the exposure (critical control variable) to the substance(s) (7). This is dependent on both understanding the effects of exposure, and the ability to measure pollutant exposure.

There is evidence that the introduction of novel entities is increasing globally, due to the increase of chemical production in the global economy, increased intensification of chemical production, and the ongoing transition to synthetic materials from natural materials (7).

1.2 Genetically Modified Organisms as Novel Entities

Genetically modified organisms can be broadly defined as any organism in which the genetic material has been altered in a way that would not occur by mating and/or natural recombination (9). Genetic modification in the context of GMOs is considered here as distinct from naturally occurring genetic changes, arising as a result of mutation, recombination or other naturally occurring processes, and is also considered distinct from selective breeding.

Traditionally, the method of modifying organisms to enhance desirable qualities was selective breeding. Desirable qualities are identified, the individuals displaying desirable qualities bred, and the offspring displaying the same desirable qualities selected for further breeding. Over multiple generations, involving multiple desirable traits and complex breeding strategies, a population is produced with distinct qualities. In traditional selective breeding, genetic changes are observed, and are the result of human selection, but are not considered GMOs.

In the twentieth century as computing and genetic sequencing were developed and increased in power, traditional selective breeding could be enhanced by computer-assisted breeding. This involved the identification of genes conferring a desirable quality, identification of organisms containing the desired gene or gene variant (allele), breeding of selected organisms, and identification of offspring for subsequent breeding according to genes or gene variants present. Although this methodology is more targeted than traditional selective breeding, involving the direct selection of specific genes and genetic variants, such organisms are also not considered GMOs.

In the 1940s, genetic mutations were induced in plants by exposure to radiation or chemicals and could be selected by plant breeders when displaying desirable characteristics (11). Genetically modified organisms as we consider them today were developed in the mid-to-late-20th century, with the first published case in 1973, involving the transfer of an antibiotic resistance gene from one bacteria strain to another (12, 13). In 1974, viral DNA sequences were integrated into mouse genomes, giving the first gene-edited animals (14). In 1983, the tobacco plant was genetically modified to contain an antibiotic resistance gene, followed by the first commercialised gene-edited plant – tobacco plants modified to contain a disease-resistant gene (15). Genetically modified food crops were commercialised in the 1990s, beginning with the FlavrSavr tomato (15, 16). Bacteria have also been developed to produce pharmaceuticals and were the first examples of commercialised genetically modified organisms (17).

1.2.1 Genetic Modification Techniques

To genetically modify an organism, three decisions must first be made so that the gene-editing protocol can be designed:

1. The Approach – Is the aim to remove DNA, to mutate DNA or to introduce new DNA?

2. The Tool – What is the mechanism performing the modification?
3. The Method – How is the DNA and/or modifying tool introduced to the cells they are intended to modify?

The gene-editing approach applied is dependent on the purpose of genetic modification. This can involve the introduction of new genetic material (insertion), either from the same species or from a different species, the modification of existing genes, or removal of portions of genetic material (deletion). Gene function can be disrupted by removal of all or part of the gene, or by a specific mutation which disrupts the expression of the gene or translation of the gene into a protein product. Gene disruption (knock-out) can also be achieved by inserting new DNA in the gene, therefore disrupting the expression and translation of the gene into its product (18).

A common use of insertion is to insert a 'marker' gene in a target region. Markers are genes encoding fluorescent proteins, such as the green fluorescent protein originally from the jellyfish *Aequorea victoria*, inserted into the genome. The fluorescent gene can be inserted alongside another gene, to create a 'tag', wherein the protein product is attached to the fluorescent protein, and thus shows the location of the protein within a cell or organism. Alternatively, the fluorescent gene can be combined with a 'promotor'; a portion of genetic material influencing the location of gene expression e.g., the eye. Under the control of the location-specifying promotor, the marker gene is expressed in that location, so enables identification of genetically modified organisms. This can be combined with other approaches, so for example, a marker gene is inserted into another target gene, resulting in disruption of the target gene, and providing a marker to indicate the organisms for which the target gene has been disrupted.

The earliest examples of gene editing depended on the assembly of circular DNA (plasmids) by enzymes, which could then be taken up by bacterial cells. The novel genes remained in the plasmid DNA, rather than being inserted into another section of DNA within the organism (13, 19). In the first genetically modified mice, gene editing was not targeted and relied on a DNA virus to integrate into the genome (14). While effective, the former method is limited to organism containing circular DNA, and the latter is inefficient and limited by the type of DNA it is possible to integrate into a host genome, in addition to a lack of control over where the novel DNA is inserted.

More targeted tools for genome modification were first developed in the 1980s using a programmable zinc finger nuclease (ZFNs), a protein which could guide a DNA cutting enzyme to the site of interest (20). In 2011 a similar, but simpler method was developed, known as transcription activator-like effector nucleases (TALENs) (21). Since 2013, a technique based on the CRISPR/Cas9 bacterial immune system has been used as a tool for gene editing (22, 23). Targeted gene editing methods have a major benefit over random-insertion methods, in that they can be used to specifically modify a target gene and with greater efficiency. CRISPR gene editing in particular has the further benefits of high adaptability but is also simpler and far cheaper than other targeted genetic modification tools.

CRISPR/Cas9 gene-editing is based on the immune system of the bacteria *Streptococcus pyogenes*. In its simplest form, the system comprises of two parts – the Cas9 enzyme and a small section of genetic material, the guide RNA (gRNA). The Cas9 enzyme can cut DNA, which is then repaired by the cell's DNA repair mechanisms. DNA repair is not always successful and can result in mutations at the point where the DNA has been cut. Mutations can disrupt the function

of the target gene, and thus generate a gene knock-out. For the Cas9 enzyme to cut the DNA at the target site, it requires a guide RNA. The guide RNA contains the genetic sequence of the target site, and thus directs the Cas9 enzyme to the target site to cut the DNA. A further DNA component can be added, termed the 'repair template'. The repair template consists of DNA matching either side of the cut site, flanking a section of novel DNA, such as a marker or another gene. After the DNA is cut the repair template is used by the cell to repair the cut site, thus integrating the novel gene at the cut site (22, 24).

Gene editing techniques based on mobile genetic elements termed 'transposons' have been integral in the development of gene editing in fruit flies and other insects. Transposons are DNA sequences which can replicate and re-insert into a new region of the host genome (25). Modified versions of transposons can be used to insert novel DNA into the genome in combination with a 'transposase' enzyme but are not directed to a specific site as is the case in CRISPR gene-editing. Versions of transposons known as P-elements have been developed for use in *Drosophila* fruit flies (26), and a transposon class known as *piggyBac* is in use to insert novel DNA into other insect species (27-30) including mosquitos (31), and has subsequently been developed for use in other species, including mammalian cells (32, 33).

There are many methods for introducing novel DNA (transformation) into the target cells or organisms, and the method used will depend on the organism of interest. Transformation can be achieved easily in bacteria and yeast, with the use of chemicals or the application of an electric field to open holes in the cell membrane which then allows the novel DNA to enter the cell. Plants can be transformed by a variety of methods, including the use of modified viruses or the modified bacterium *Agrobacterium tumefaciens* (34). An alternative involves the technique of 'biolistics', which involves coating small particles of gold or tungsten with DNA, then firing the particles into cells or young plants (35). Introducing new DNA into animal cells (transfection) can involve similar chemical and non-chemical methods to bacterial transformation, biolistics and viruses as in plant transformation and can also involve direct injection – termed microinjection – into the cells of interest or the organism at the earliest embryonic stages.

1.2.2 Applications of Genetic Modification

There are a multitude of applications for genetic modification and uses of genetically modified organisms. The use of GMOs has often been controversial, particularly when intended for human or animal consumption. However, the uses of GMOs are far more wide-ranging than applications in food crops and more recently, livestock. Since the early 2000s, genetic modification has been explored as a method to control the spread of insect pests and some suggestions are that genetically modified bacteria could be used to sequester and process environmental contaminants in bioremediation efforts.

GMOs are also essential in research. Genetic modification can be used to knock-out or otherwise modify target genes in model organisms, to examine their function (18, 36, 37). This is of particular use in research of the genetic basis of diseases, such as Parkinson's, Alzheimer's, and many cancers. Other applications of genetic modification in research

include investigating the role of certain genes, gene products and gene pathways in the organism development, understanding the formation of proteins and other gene products, whole organism function and behaviour (18).

GMOs are considered as part of the 'novel entities' planetary boundary, but their contribution to the planetary boundary and Earth System processes more generally are not fully defined. Part of the issue is that the term 'genetically modified organism' encompasses a huge range of organisms, gene editing techniques and applications.

1.3 Aims

Genetically modified organisms have the potential to act as novel entities, as they are defined within the planetary boundaries' framework. Yet, they remain unquantified as a planetary boundary. This report aims to explore a planetary boundary for GMOs, as follows:

1. Explore and discuss the environmental impacts and potential future environmental impacts of GMOs, whether beneficial or adverse.
2. Discuss the potential for GMOs to act as novel entities, as defined within the planetary boundaries' framework.
3. Establish how a planetary boundary for such novel genetic entities can be defined and quantified.

In the following sections, the above aims will be discussed about the uses of GMOs in crops, livestock and pest control, with brief discussions of other applications in bioremediation, medicine and research.

2 Crops

Food security has long been a concern of governments, and more so with the increasingly prevalent effects of anthropogenic climate change and rapid global population growth. A key question to address therefore is that of how to grow enough food, and how to do so sustainably. Much of the focus on providing solutions for food security has been on the development of new crop varieties.

Traditionally, new crop varieties were generated by selective breeding; the process of selecting parents with desirable qualities, such as large fruits and hardiness certain weather conditions, breeding them, and then selecting the offspring which also displayed those desired characteristics, and continuing to breed those. Over many generations, new crop varieties are developed with higher yields and greater suitability to its environment. However, this process is slow and imprecise, relying on natural variation in the crop, or on the appearance of random mutations which, often, are not favourable.

In 1996, the first genetically modified crops containing inserted genes were sold commercially. Today, 425 genetically engineered varieties of 32 crops have had approval for cultivation or import in 44 countries, either for consumption or for animal feed (38) (figure 1). In contrast, of the 250,000 crops varieties available, approximately 7500 (3%) are currently in use, and of the 150 species cultivated for food, only 12 provide most of the global food supply (39).

GM crop traits typically fall under the categories listed in table 2. The combination of multiple traits in a single crop variety is termed 'stacking'. The most common trait in cultivated GM crops is herbicide tolerance, accounting for 47% of GE crops grown worldwide, followed by stacked herbicide tolerance and insect resistance, accounting for 41% GM crop cultivation (38, 40, 41). Single trait insect resistance accounts for 12% GM crop cultivation. Disease resistance, abiotic stress tolerance, modified growth or yield and modified product quality have been applied in far fewer varieties and are cultivated on much smaller scales. Four crops make up over 98% of all GM crops cultivated globally; soybean (50%), maize (30.7%), cotton (24.8%) and canola (5.3%).

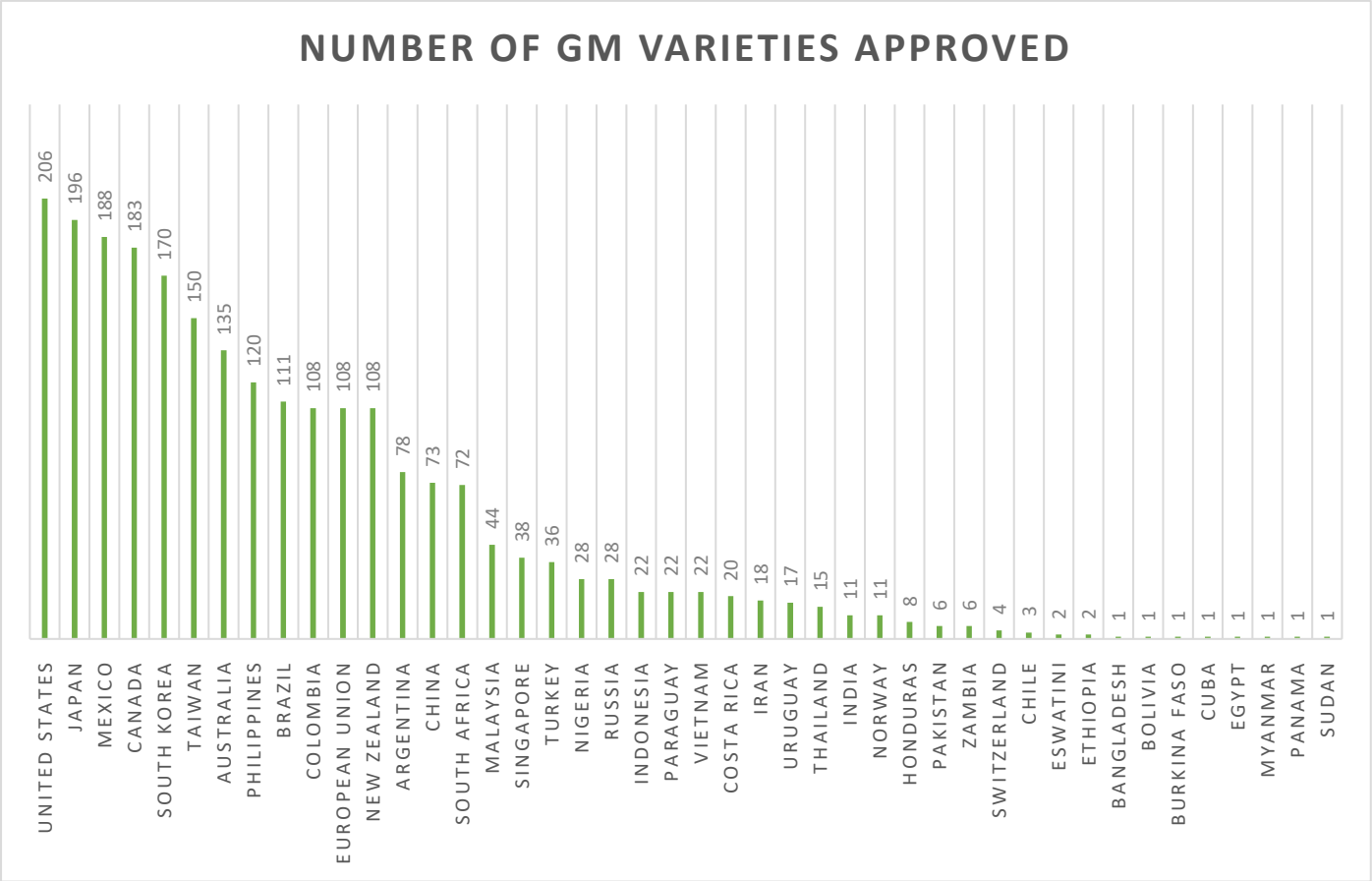

Figure 1: Total number of GM crop variety approvals by country, according to 2020 ISAAA data. Approvals are for either cultivation or import of the crop, for use as food or as animal feed. Includes discontinued varieties (38).

Trait	Function
Herbicide Tolerance	Production of a modified enzyme which is not affected by herbicides, allowing continued plant function and growth after the application of herbicides. Weed plants are susceptible to the application of herbicide and thus can be controlled.
Insect Resistance	Production of compounds targeting a specific insect pest, reducing damage and death caused by the insect pest and increasing amount and quality of harvested yields.

Disease Resistance	The plant produces a component of the target pathogen, e.g. virus coat proteins. The presence of the pathogen component triggers an immune response, similar to that of a vaccination. Alternatively, the plant produces a compound inhibiting the function of a specific pathogen. Reduces crop loss to disease, increasing the amount and quality of harvested yields.
Abiotic Stress Tolerance	Modification of genes or pathways involved in the plant response to abiotic stress (e.g. drought, flooding, extreme temperatures, salinity). Alternatively, novel genes introduced for the production of compounds which can continue to function in the presence of a stressor, or prevent damage from the stressor.
Modified Growth or Yield	Modification of pathways involved in overall plant or product growth.
Modified Product Quality	Changes to the production of specific components of the crop plant, for example by increasing production of a desirable component, or decreasing production of unnecessary or undesirable components.

Table 2: Summary of crop traits achieved by genetic modification and the mechanisms by which the traits can be achieved, reviewed in (38, 42).

Based on the traits listed in table 2, GM crop traits can be separated into two categories:

1. Traits which reduce crop loss – herbicide tolerance, insect and disease resistance, abiotic stress tolerance.
2. Traits which modify an aspect of the final product – modified growth or yield, modified product quality.

2.1 Benefits of GM Crop Cultivation

The benefits of GM crop cultivation are dependent on the trait(s) and the variety that is grown.

The ISAAA is a not-for-profit group promoting the use of GM crops, in particular for resource-poor small-scale farmers in developing countries. They promote the use of GM crops to improve crop yields and argue that their use improves agricultural sustainability. In 2018 the ISAAA estimated that the adoption of GM crops has reduced agricultural land use by 183 million hectares, reduced pesticide use by 8.2%, and reduced CO_2 emissions by 27.1 billion kg between 1996-2016 (40, 43).

2.2 Risks of GE Crop Cultivation

Despite the benefits, the growth and use of GM crops remain controversial, and there is a substantial body of research investigating the risks associated with the adoption of GM crops. In the following section, three potential issues are discussed and evaluated; transgene escape, expression of novel compounds, and associated novel entities.

2.2.1 Transgene Escape

Transgenic crops can escape their intended cultivation areas, either through the dispersal of pollen and the generation of hybrid offspring or through seed dispersal. GM contamination can occur where GM crops are unknowingly mixed with non-GM crops either as the result of an escape event or through the distribution of GM seed as non-GM. Over 396 cases of GM crop escape, hybridisation and contamination have been documented, in at least 63 countries worldwide (44, 45). There are too many cases to detail here, but some examples and general patterns of transgene escape are discussed. The examples discussed below, and further examples of GM crop escape and hybridisations events are summarised in tables 3 and 4.

GM crops may hybridise with different GM and non-GM varieties of the same species, or with closely related cultivars, landraces and species, both wild and domesticated. Hybridisation generates 'hybrid offspring' combining traits of the two parents, and may include the novel genetic trait, or 'transgene'. Over generations, the offspring of these hybridisation events can spread, and the transgene becomes 'fixed' in a population – that is, the gene is present in all individuals in a given area – also termed 'introgression'. Hybrids between different GM cultivars may contain a new combination of stacked traits.

DEFINITIONS:

SPECIES – A GROUP OF SIMILAR INDIVIDUALS WHICH CAN REPRODUCE SUCCESSFULLY WITH EACH OTHER. OFTEN THEY ARE REPRODUCTIVELY ISOLATED FROM OTHER SIMILAR SPECIES.

VARIETY – A GROUP WITHIN A SPECIES, DISPLAYING CHARACTERISTICS DISTINCT FROM THOSE OF THE PARENT SPECIES. VARIETIES ARE ABLE TO INTERBREED WITH DIFFERENT VARIETIES OF THE SAME SPECIES.

LANDRACE – LOCAL VARIETIES OF A DOMESTICATED PLANT SPECIES WHICH HAVE DEVELOPED A DEGREE OF ADAPTATION TO THE ENVIRONMENT IN WHICH IT IS GROWN. OFTEN HAVE MORE GENETIC DIVERSITY THAN CULTIVARS.

CULTIVAR – SIMILAR TO VARIETIES AND LANDRACES, BUT DO NOT OCCUR NATURALLY. CULTIVARS ARE BRED BY HUMANS, OFTEN TO IMPROVE CERTAIN DESIRABLE CHARACTERISTICS.

Seed dispersal can occur through the unintended spreading of seed often during transportation, by dropping of seed at the cultivation site and emergence in subsequent seasons when the area is no longer planted with the GE crop, or by natural seed dispersal mechanisms – wind or animals. These escaped plants are known as 'volunteers'. Seed dispersal increases the likelihood of hybridisation, as GM seeds are mixed with or are in closer proximity to other varieties and cultivars with which it can breed.

GM crop escape events have been documented going back to a case in Oaxaca, Mexico in 2001, when herbicide-tolerant maize crops were found to have hybridised with non-GM cultivars growing nearby (46). The case was

controversial and a study testing samples taken from the same area in 2003 and 2004 did not find evidence of GM maize hybridisation (47). Subsequently, retesting of the original samples confirmed the presence of transgenes in 11 of 60 non-GM maize fields, along with evidence of new GM hybridisation cases in Mexico (48-50). A case study in Uruguay tested samples from five side-by-side GM and non-GM maize fields, finding the presence of insect resistance transgenes in three of the five fields (51). Similar findings have been reported in herbicide-tolerant and insect resistant maize grown in Colombia and South Africa (51, 52). In the latter case, the authors found that seed sharing and seed recycling among farmers was commonplace and was a major contributor to the transgene escape events.

Multiple cases of unintended seed distribution during transportation have contributed to the escape events of GM canola (*Brassica napus*). GM canola volunteers have been found along verges and non-GM fields in the USA and Canada, along railways lines in Switzerland, and near ports in Japan (53-57). Further investigation into the cases in Japan revealed the source was imported GM canola, and also identified hybrids of herbicide-tolerant *B. napus* and *B. rapa*, which contained the gene for glyphosate tolerance (54).

Researchers have found evidence of transgene flow between cultivated GM cotton and wild landraces, raising concerns over the genetic diversity of wild cotton populations. In an analysis of eight metapopulations, evidence of transgene flow into wild cotton was identified in four (58). Gene flow from insect-resistant GM cotton to non-GM cultivars has also been identified, suggested to be the result of seed mixing and interbreeding (59). In the latter case, insect-resistant cotton was identified up to 2km from the nearest GE site.

Several high-profile cases of GM escape have occurred, not from cultivation, but from field trial sites. Volunteer herbicide-tolerant wheat plants were identified in Washington State, Oregon and Montana up to 16 years after field trials had ended (60-62). The incidence in Oregon was reported to have triggered a decline in the importation of US wheat into Japan and South Korea, over contamination concerns.

Another notable case from the USA was that of herbicide-tolerant creeping bentgrass, planted on golf courses as part of a field trial. Gene flow was identified up to 21km away from the test site, likely due to the dispersal of pollen by wind and hybridisation with non-GM plants (63). A follow-up study 13 years after field trials identified that GM creeping bentgrass had become established in the environment and had hybridised both with a closely related species, redtop (*Agrostis gigantea*), and with a species of another genus, rabbitfoot grass (*Polypogon monspeliensis*) (64, 65). In both of these cases, the hybrids were identified as herbicide-tolerant as a result of the transgene. It is unclear to what extent the escape and inter-species hybridisations will be problematic, although the concern is that the spread of herbicide tolerance into non-GM populations and inter-species hybrids will result in novel weed species, difficult to control. Whether or not this is the case, it is widely considered that herbicide-tolerant creeping bentgrass and transgenic hybrids have escaped irretrievably into the environment (65, 66).

Herbicide-tolerant rice hybrids have occurred with an agricultural weed variety, red rice, in Italy and Brazil (67, 68). In Italy, tolerance to the herbicide Clearfield® was found to have persisted in red rice populations over multiple generations, suggesting that the transgene had become introgressed. Introgression can not only increase the chances

of herbicide-tolerant weed populations becoming a persistent agricultural pest, but Busconi (67) suggests that Clearfield tolerant red rice may now be in the cultivated rice seed supply, increasing the likelihood of further spread.

Multiple cases of transgene escape relate to crops which do not, or did not at the time, have approval for cultivation (e.g. LLRICE, Bt63 rice, Bt10 maize, papaya, creeping bentgrass). Evidence from some cases indicates transgene flow originated from the test sites, however, concerns have also been raised about the unregulated sale and use of GM crops (45). In many cases the exact origin of the volunteer or transgene is unknown.

It is clear from the examples of transgene escape discussed here, as well as many other cases documented in the GM Contamination Register and in other reviews that transgene escape is a widespread phenomenon where GM crops are planted. Certain factors increase the risks of transgene flow, namely ease of pollen and seed dispersal, transportation before processing, and proximity to other plants with which hybridisation can occur. Persistence of transgenes in the environment is not guaranteed but has been observed, most notably in the cases of creeping bentgrass and red rice.

It is generally considered that crop plants are less able to survive outside of cultivation, being less 'fit' than feral or wild landraces. Transgenes can confer advantages over other landraces, by enabling resistance to insect pests or abiotic stress, or through tolerance to herbicides, to which they may be exposed directly or through herbicide drift. Transgenes can therefore increase the fitness of a GM crop outside of cultivation, increasing the chance it will survive and spread, and if introgressed into a weed, may increase its persistence and invasiveness. An example of this is herbicide-tolerant canola, identified in Switzerland. Glyphosate tolerant canola was found growing along railway lines, where the seed had been dropped unintentionally during transportation before processing. Weeds were controlled in these areas by spraying glyphosate-based herbicide, thus removing non-tolerant weeds, but the escaped tolerant canola was still able to grow. In this case, the presence of the glyphosate tolerance transgene allowed the escaped crop plants to survive and thrive in an environment they were not intended for, and in effect was selecting for plants containing the transgene (57).

In the Swiss canola example, GM-HT escape was identified after the glyphosate-based herbicide was sprayed and survivors noticed. Identification of survivors after herbicide application has been a common way for escape events to be identified (see also three cases of HT wheat escape in the USA). This illustrates an issue with the identification of GM escapes – often they are noticed by chance. Identification and monitoring GM crop escapes is a fundamentally intensive and difficult task, which would be impossible to do in every area where GM crops are regularly planted. Furthermore, even where escape events are identified and monitored, such as the GM-HT bentgrass case in Oregon, it may not be possible to eradicate the now feral transgenic plant. In the case of herbicide-tolerant creeping bentgrass, many consider transgenic plants to be permanently established in the environment (65, 66).

Given the issue of transgene flow, there have been numerous suggestions for methods to prevent or minimise the risk of escape. These might include biological methods, which limit the ability of the modified plant to hybridise with other plants, such as a genetic modification to render males and/or females sterile, prevent flower opening, or to confer clonal seed production (seed production without fertilisation). Alternatively, GM could be limited to crops which are

already sterile, such as banana, cassava and potato (69). Another suggested method involves genetic modification to increase glyphosate-resistant maize susceptibility to the herbicide micsulfuron – to which conventional maize is resistant – therefore providing a method to selectively remove GM maize (70).

Studies have also found that the use of physical barriers can reduce pollen-mediated gene flow. One such study found that the use of nets in combination with another crop separating GM fields was the most effective in reducing pollen-mediated gene flow, compared to nets and separation crops individually (71). Transgene flow is also heavily dependent on farmer practices, indicating the need for greater training and guidance for farmers using these crops, to understand the risks and prevention strategies required GM crop use (52, 72, 73).

Why is all of this a problem? As discussed above, uncontrolled growth of transgenic plants can result in weed plants which are increasingly difficult to control. More invasive agricultural weeds decrease agricultural yields, and invasive weeds can affect biodiversity, another of the planetary boundaries. Another important aspect of this is the genetic diversity of hybridised and introgressed plants, where a selected for transgene confers greater fitness, it is more likely to reproduce and can better persist in the environment where local landraces previously existed, thus changing the biodiversity and genetic diversity of the species in the wider environment. Landraces and non-domesticated varieties can be sources of genetic diversity for plant breeders in generating new varieties capable of growing in a changing climate.

The extent of the risk of transgene escape to genetic diversity and biodiversity is debated by some. In the case of GM maize, Parrott (74) argues that the risks posed by transgene flow from GM maize into non-GM landraces are low; that despite gene flow between cultivated varieties and teosinte populations, they have remained distinct. Parrott claims that the greatest risk to the survival of traditional maize landraces is that of farmer-migration to cities, not the adoption of GM-crops.

It is also necessary to consider transgene escape from an ethical standpoint – if transgenes escape so readily, we must consider whether humanity should be introducing such novel genetic entities into the wider environment, where independent from us they are capable of wider ecological interaction and evolution.

There is a clear risk of transgene escape from GM crops, which has the potential to be hugely disruptive to weed management in agricultural and wider settings, to the genetic diversity of related landraces, and more generally to biodiversity in the wider ecosystem. It is unclear to what extent escapes which have already occurred have had a significant negative impact in these areas, as identification and monitoring of escape events is difficult. There are numerous predictions of significant negative impacts, and where possible escape events must be studied to establish the risks posed. From the information currently available, it is not yet possible to determine whether a planetary boundary for transgene escape has been crossed, although we now have a growing idea of how it may look. More research is required to establish exactly where a boundary of transgene escape may lie, and how this contributes to an overall planetary boundary of GM crop cultivation. What is certain, however, is that transgene escape has complex and often unpredictable consequences, of unclear severity.

GM crop	Location(s) of Contamination/Escape Event	GM Event	Description	References
Alfalfa (*Medicago sativa*)	USA	Herbicide Tolerance	Feral alfalfa identified at 404 sites, of which 27% also had GM alfalfa. The suggested source was seed spillage from transportation and production.	(75)
Canola (*Brassica napus*)	Canada Japan Switzerland USA	Herbicide Tolerance	Herbicide-tolerant GM canola identified at 14 Japanese ports importing GM canola. In Switzerland, 58/79 locations along railway lines sampled had glyphosate-resistant canola present. Glyphosate herbicide was regularly used along railway lines to remove weeds, providing an advantage for escaped GM canola. Widespread GM canola escape in Manitoba, Canada. Persistence of escaped GM populations for 2-5 years with repopulation from new escape events. Contamination of non-GM seed lots by GM canola also found in Canada.	(53-57, 76)
Cotton (*Gossypium hirsutum L.*)	Colombia	Insect Resistance	Escape event or mixing of GM and non-GM cotton seeds. GM cotton plants from escaped seed up to 2km from closest GM seed.	(59)
Creeping bentgrass (*Agrostis stolonifera*)	USA	Herbicide Tolerance	Multiple studies identifying escaped herbicide-tolerant creeping bentgrass up to 21km from a controlled test site via wind-dispersed pollen and seed. Most gene flows occurred within 2km of a test site.	(63, 64, 66, 77)

Maize (*Zea mays L.*)	South Africa South Korea	Herbicide Tolerance Insect Resistance	Transgene sequences identified in non-GM leaf and seed samples from South African farmers. Seed sharing and seed recycling common practice among farmers surveyed. GM maize kernels found around ports in South Korea from maize importation. Three cases identified, each containing different GM events.	(52, 78)
Wheat (*Triticum aestivum*)	USA	Herbicide Tolerance	Three separate events of glyphosate-resistant wheat plants found growing 10-16 years after the end of field trials in Montana, Oregon and Washington state, USA.	(60-62)

Table 3: Examples of GE crop escape and contamination events.

GM crop	Location(s) of Hybridisation Events	Hybridisation	GM Event	Description	References
Canola (*Brassica napus*)	• Argentina • Australia • Japan	Wild *B. rapa* Non-GM canola	Herbicide tolerance	Evidence of introgression of herbicide tolerance gene from feral GM canola (*B. napus*) into wild *B. rapa* (global agricultural weed), selected for by use of glyphosate herbicides in Argentina. Herbicide tolerance also identified in *B. napus x B. rapa* hybrids along riverbanks after GM canola (*B. napus*) identified at ports in Japan. Low levels of pollen-mediated gene flow identified between GM and non-GM canola fields in Australia.	(54, 79, 80)

Cotton (*Gossypium hirsutum L.*)	• Colombia • Mexico	Wild cotton Non-GM cotton	Herbicide tolerance Insect resistance	Transgene flow between GM and non-GM cotton cultivars identified resulting from seed mixing in Colombia. Evidence of transgene flow in 4/8 large wild cotton populations in Mexico. Herbicide tolerance and insect resistance genes found.	(58, 59)
Creeping bentgrass **(*Agrostis stolonifera*)**	USA	Redtop grass (*Agrostis giantea*) Rabbitfoot grass (*Polypogon monsepeliensis*)	Herbicide tolerance	An interspecific hybrid between creeping bentgrass (*A. stolonifera*) and redtop grass (*A. gigantea*) and intergeneric hybrid with rabbitfoot grass (*P. monspeliensis*) as a result of pollen mediated gene flow. Hybrids were identified over four years up to 5km from planting site, 13 years after removal from the controlled site.	(65)
Maize (*Zea mays L.*)	• Colombia • Mexico • South Africa • Uruguay	Non-GM maize	Herbicide tolerance Insect resistance	Multiple studies finding evidence of GM maize hybridisation with non-GM maize in nearby fields. Hybrids found in 18-60% of sites investigated.	(48, 51, 52, 73)
Rice (*Oryza sativa L.*)	Brazil Italy	Red rice (*O. sativa L.*)	Herbicide tolerance	Evidence of gene flow from herbicide-tolerant rice (*O. sativa L.*) to weedy cultivar red rice in sites in Brazil and Italy. Herbicide-tolerant red rice samples in Italy had transgene for at least two generations.	(67, 68)
Sugar beet (*Beta vulgaris*)	France	Weed beet	Herbicide tolerance	Transgene flows from herbicide-tolerant sugar beet in a test site to weed beet.	(81)

Table 4: Examples of identified GM crop hybridisation events.

2.2.2 Expression of Novel Compounds

Production of novel compounds is one of the common methods of engineering traits in crops. Glyphosate tolerant crops produce a version of the EPSPS enzyme which is not inhibited by the herbicide, insect-resistant crops rely on variations of the *Bacillus thuringiensis* (Bt) toxin specific to the target insect pest, disease-resistant crops may produce viral coat proteins or other viral components, and plants with engineered tolerance to various abiotic stress factors often rely on compounds originally found in organisms which thrive in those conditions (see table 1). Numerous attempts have been made to untangle the potential effects of novel compounds produced by GM crops on non-target organisms (NTOs) (table 5).

The majority of studies have found inconsistent or non-significant effects of GM crops or their novel products (arising from the transgene) on microbial community abundance and diversity (82). Zhaolei *et al*. (83) found no evidence for an effect of the Cry1Ac protein (produced by insect-resistant GM crops) on soil bacterial, fungal and archaeal community composition and population size, among numerous other studies which have also not observed significant effects (84-95). Some studies have found significant effects on microbial communities, for example, Li (96) found that rice with stacked insect resistance and drought tolerance did have an effect on bacterial community composition and decreased bacterial population size, and Liu (97) found bacterial population sizes decreased while fungal population sizes increased as a result of herbicide-tolerant soybean. Effects on microbial communities have also been noted for maize and aubergine insect-resistant crops (82).

Other non-target organisms may also be affected by GM crop cultivation, for example, Mina (98) found increased numbers of nematodes, springtails and ants in insect-resistant cotton fields. Other studies have not found any effects on insect mortality or abundance (99-102). Non-target aphids feeding on insect-resistant soybean sap were found to accumulate the *Bt* toxin Cry1Ac, prompting concerns by the authors that the toxin could accumulate in beneficial aphid-predators (103). This remains unclear, however, and more research would be needed to establish the impacts of *Bt* toxin accumulation in the field at higher trophic levels.

Health effects of GE crop consumption could also be considered within the category of expression of novel compounds. The potential for negative health consequences of GM food consumption has long been a highly controversial area of research and debate. One argument states that GM crops are regularly consumed by millions, without any apparent adverse effects (104). The vast majority of studies investigating toxicity related consumption of GM food and feed have found no evidence of adverse effects (105), and where an adverse effect has been observed, it is minor (104). Published studies which have claimed to have found clear evidence of health risks posed by GM food consumption have generally received harsh criticism by scientists in the field, as misleading and poor quality (104).

GE Crop	GE Trait(s)	Description
Canola (*Brassica napus*)	Herbicide tolerance	Decreased bacterial population size, increased fungal population size (97).
Canola (*B. napus*)	Insect resistance	Bt toxin present in non-target aphids (103).
Canola (*B. napus*)	Modified product quality (High methionine)	No effect on nitrogen-fixing or ammonia oxidising bacteria community composition (85).
Cotton (*Gossypium hirsutum* L.)	Herbicide tolerance	No effect on the bacterial population size (91).
Cotton (*G. hisutum* L.)	Insect resistance	Changes to soil biochemistry; increased dehydrogenase enzyme activity. Increase in nematode, springtail (Collembola) and ant (Hymenoptera) population size (98). No toxicity observed in target or non-target insects fed with leaves and pollen (102). No effect on bacterial population size or diversity (88). No consistent differences in cultured bacteria (93, 94).
Eucalyptus (*Eucalyptus sp.*)	Abiotic stress tolerance (salinity)	No effect of cultured bacteria, no effect on lettuce seedling growth (86).
Maize (*Zea mays* L.)	Herbicide tolerance	Bt toxin from decaying leaves persisted in soil up to 40 days (106).
Maize (*Zea mays* L.)	Insect resistance	No effects observed on non-target insects (99, 100). No change in community composition of endophytic bacteria (84). No effect on cultured aerobic bacteria (89). Decreased abundance of nitrogen transforming bacteria and archaea, but no overall change in community composition (107). Mathematical models show the mortality rate for non-target butterflies and moths is very low and low, respectively (108).

Poplar (*Populus sp.*)	Insect resistance	Increased mortality of target insects, no increase in mortality of one non-target insect species fed with leaves (101).
Potato (*Solanum tuberosum L.*)	Modified product quality (amylopectin production, cyanophycin production)	No effects on bacterial or fungal population size (90). No effect on bacterial population diversity (92).
Rice (*Oryza sativa*)	Insect resistance/drought tolerance (stacked)	Decreased bacterial population size and changes in community composition, no effect on fungal population size, lower soil pH (96).
Rice (*O. sativa*)	Insect resistance	Decreased abundance of methanogenic archaea (109). Increased diversity of methanogenic archaea (110). No effect on non-target insect *F. candida* (111). Decreased abundance of non-target pest insect due to lower release of volatiles from damage caused by target insects (112).
Rice (*O. sativa*)	Drought tolerance	No effects on bacterial population size or community composition (95).
Wheat (*Triticum aestivum*)	Disease resistance (mildew)	Inconsistent effects on beneficial bacterial abundance, no significant effect overall (87).

Table 5: Examples of research into potential effects of GE crops on non-target organisms, including soil microbes and non-target insects. Note, results between studies are not directly comparable due to variation in methods used to identify and quantify non-target effects.

2.2.3 Associated Novel Entities

Modern agriculture relies heavily on chemicals, in the form of herbicides, insecticides and other pest controls, and fertilizers. The sustainability of heavy reliance on these chemicals has been questioned, with many researchers, campaign groups, and regulatory bodies suggesting they may be creating long-term damage. The issue of chemical use in agriculture is highly relevant to the GM crop debate, as the most common GM trait in crops is herbicide tolerance – the ability to continue function in the presence of a herbicide. GM crops have been modified for tolerance to a variety of herbicides, the most common of which are glufosinate and glyphosate (figure 2), both in terms of varieties available and in area planted (38, 41).

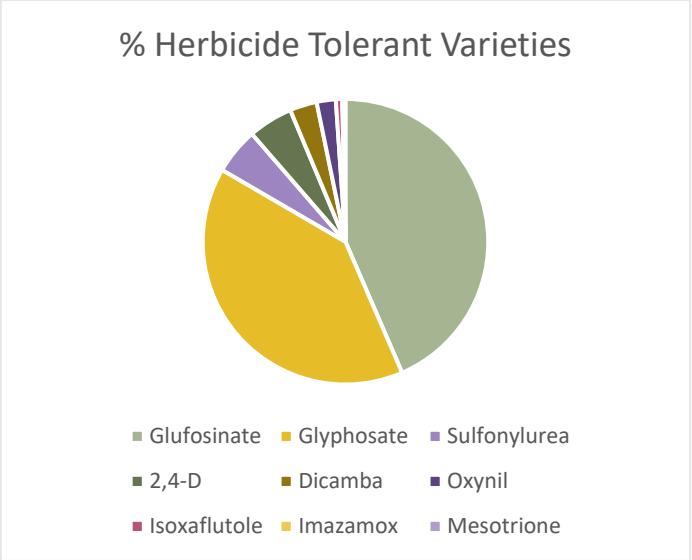

Figure 2: Percentage approved varieties with herbicide tolerance, data from ISAAA (38).

Glyphosate is a 'universal' and 'systemic' herbicide, targeting the shikimate pathway present in plants, bacteria and fungi. It binds to the EPSPS enzyme, preventing growth, causing damage from free-radicals, and increasing exposure to pathogens. The vast majority – approximately 90% – of global glyphosate use is in agriculture (113). It is also used in public spaces to control weeds, such as along river banks and in public parks, in waterways to eliminate invasive aquatic plants and is also used in private gardens (114). In non-GM agriculture, the glyphosate-based herbicide is sprayed before sowing to remove cover crops or to control weeds, and after harvesting. In GM agriculture, glyphosate can be applied after sowing for ongoing weed control. Increasingly, it is used to accelerate the desiccation of cereal crops before harvesting. The use of glyphosate in weed control reduces the need for tilling, decreasing fuel use and emissions from heavy machinery, and decreasing soil erosion (115). In combination with GM glyphosate-tolerant crops, glyphosate herbicide offers a simpler and more effective method of weed control than selective herbicides and offers farmers savings in time, labour and costs compared to traditional weed control methods (116). For these reasons, some argue that glyphosate use has an overall benefit to the environment (115, 116).

As a simple method of weed control, glyphosate-based herbicide use has increased dramatically since it was first introduced in the 1970s, and increased more rapidly since the introduction of glyphosate-resistant GM crops in the 1990s; rising from 51 million kilograms in 1994 to 747 million kg in 2014 (113) (figure 3). At the average rate of application (1.5-2kg per hectare), the volume of GBH used in 2014 is estimated to have covered 22-30% of cultivated cropland worldwide (113). In a review of global glyphosate use, Benbrook (113) concluded: "no pesticide in history has been sprayed so widely".

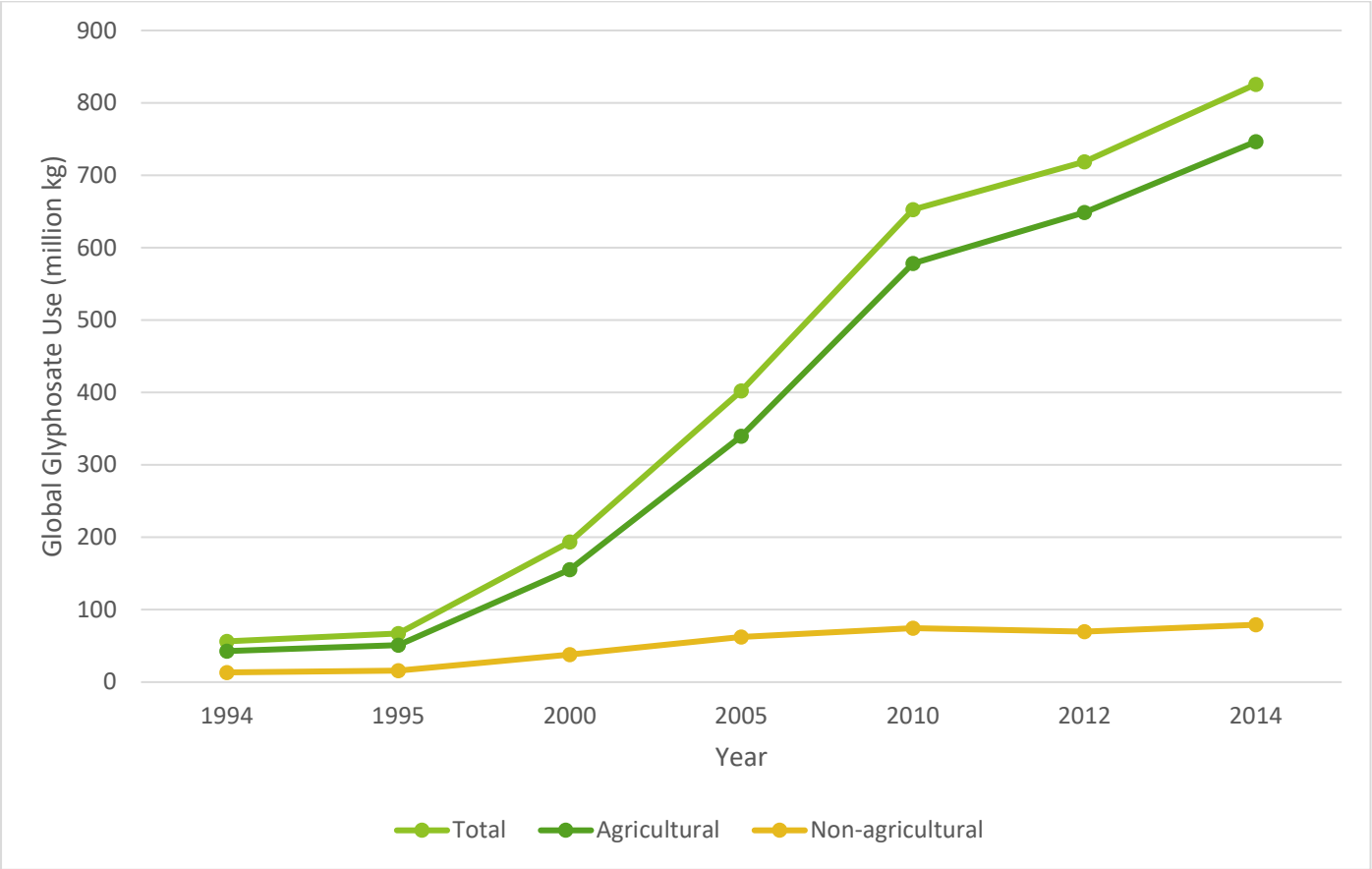

Figure 3: Global application of glyphosate from 1994-2014, from data in (113). Total glyphosate use is equivalent to the figures for agricultural and non-agricultural combined.

As discussed above, there are concerns over transgene flow as a mechanism for the development of herbicide-tolerant weeds harder to control. Tolerance and resistance to herbicides including glyphosate in non-target plants can also evolve by natural selection, without the need for transgene flow, and is more likely as the herbicide in question is adopted over a wider area. At present, known mechanisms of glyphosate resistance outnumber the mechanisms described for any other herbicide (117) and the increase in adoption of GM-HT crops has led to shifts in the composition of weed communities towards less-sensitive and more resistant weeds, in turn leading to more frequent and more intensive GBH applications (113). The increase in resistant weed populations is predicted to have significant impacts on biodiversity and agricultural productivity.

With the widespread adoption of glyphosate, both in GM and non-GM agriculture, the safety of glyphosate-based herbicide has come into question. Concerns have been raised over the impacts of glyphosate and other components (adjuvants) on non-target organisms and wider ecosystem function. Establishing the toxicity of adjuvants is made more complex by the fact that as 'corporate secrets' they are often not declared on product labels (118, 119). Nevertheless, some adjuvants have been determined to have higher toxicity than glyphosate itself, or contribute significantly to the toxicity of glyphosate, particularly for aquatic organisms (120, 121).

In a brief for the UN, Stefan Schwarzer reviewed the environmental impacts of glyphosate herbicide use (122). He reports that the intensive use of glyphosate has resulted in the development of herbicide resistance, disruption of soil microbial communities, plant die-off increasing the presence of phosphorus and nitrogen salts in the soil, and toxic

effects of glyphosate-based herbicides were found in algae, plants, fish, a variety of invertebrates and mammals. Furthermore, the removal of weeds and other non-target plants reduces the availability of habitat and food sources for insects, with knock-on effects on bird and mammal populations. Of particular concern were the effects of glyphosate on two 'keystone' species, so-called because they provide essential ecosystem services. Glyphosate herbicide exposure leads to lower activity, weight and reproductive capability of adult earthworms, reduced hatching and decreased numbers of juveniles and cocoons (122, 123). In bees, essential pollinators, glyphosate exposure at ecologically relevant levels (5mg/L) has been found to reduce the abundances of gut bacteria and increased susceptibility of bees to pathogens (124). Glyphosate exposure has also been found to decrease survival of honey bee larvae, and at sub-lethal levels reduces larval growth (125).

Glyphosate and its adjuvants can accumulate in sprayed crops as well as persisting on their surfaces, raising concerns for the impacts on human health arising from the consumption of the harvested crop. In toxicity tests of glyphosate herbicide formulations on human cells, effects on cellular respiration and membrane integrity were observed at concentrations of 1-3ppm (126). Glyphosate-based herbicides have been shown to disrupt endocrine mechanisms in liver cells at concentrations of 0.5ppm, and toxic effects at 5ppm (127). Glyphosate herbicide exposure has been found to disrupt embryo neural and cranial development in studies of chicken and amphibian embryos (128). The presence of glyphosate herbicide adjuvants also increases toxicity compared to glyphosate alone, potentially by increasing cell penetration and stability of glyphosate, and thus amplifying changes to metabolism in cells (127) and the adjuvant POE-15 can induce necrosis in cells (126). There has also been significant discussion of whether glyphosate herbicides are carcinogenic (122); the World Health Organisation has designated glyphosate as a 'probable carcinogen' with links drawn between glyphosate exposure and non-Hodgkin lymphoma (129), although some studies have not found the same correlation (130).

Exposure to glyphosate and its herbicide formulations can cause wide-ranging detrimental environmental effects. The level to which target and non-target organisms are exposed to glyphosate-based herbicides is heavily dependent on its distribution and persistence in the environment. Glyphosate reaches soil by direct application, washing off from plant surfaces by rain, air-borne drift, precipitation, root exudation, and decomposition of treated plant material (118). Glyphosate is often touted as 'safe' once in the soil, as it is rapidly absorbed into soil particulates and degraded to its main metabolite, AMPA, by soil bacteria and fungi (figure 4) (131). Many studies have since investigated the extent to which glyphosate and its metabolites persist in soil. A study in Argentina sampled cultivated soil at 16 agricultural sites, finding concentrations of glyphosate ranging from 35-1502µg/kg, and AMPA concentrations ranging from 299-2256µg/kg. Glyphosate was detected at two control sites, where it had not been applied, likely to be due to herbicide drift. In streams, glyphosate was found up to concentrations of 7.6µg/L, and AMPA up to 2.3µg/L. Glyphosate and AMPA were also detected in suspended particulate matter (up to 562.8 and 210.4µg/kg, respectively), and in sediment (221.2 and 235µg/kg, respectively). The authors conclude that soil runoff is responsible for the movement of glyphosate adsorbed into soil particulates in streams, wherein it can biodegrade to AMPA, in addition to forming complexes with metal ions such as copper and iron (132). Runoff has led to glyphosate contamination of seawater, wherein it persists for up to 330 days, depending on light and temperature conditions (133).

Persistence in soil exposes soil microorganisms to glyphosate. A review by the Soil Association reported conflicting results in a review of the effects of glyphosate on soil microorganisms.

Microorganisms resistant to glyphosate can use it as a source of phosphorus, nitrogen, and carbon, and show increased abundance in the presence of glyphosate. The effects of glyphosate and glyphosate-based herbicides appear to be species- or strain-specific; one study found that while proteobacteria abundance increases, acidobacteria abundance decreases, and the fungus *Asperigillus nidulans* experienced toxicity effects well below the recommended application rates. Lab studies have identified shifts in microbial community composition because of increased carbon availability from decaying roots after glyphosate application. Repeated glyphosate herbicide application results in shifts in fungal species towards more resistant (although this finding has been inconsistent). Recovery from glyphosate-induced soil microbiome disruption is less well-understood; some studies have found that disruption is transient, however more research is needed to understand the long term effects of repeated GBH exposure on soil microbial communities (123).

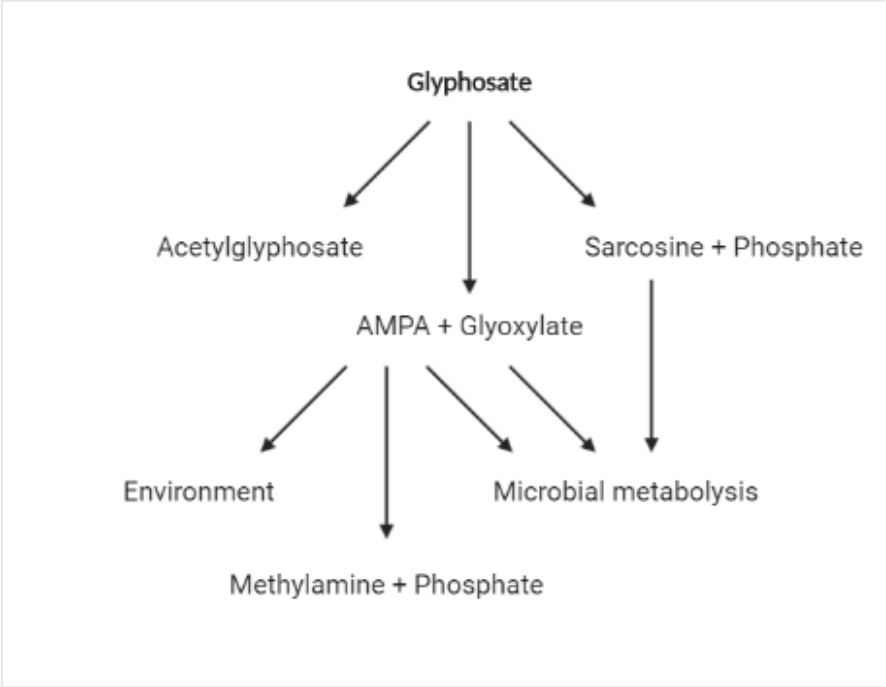

Figure 4: Metabolic pathways for glyphosate degradation by soil bacteria and fungi. Adapted from Zhan et al. 2018 (131).

Glyphosate microbial toxicity is not limited to soil microorganisms. Two food microorganisms, relied upon in the dairy industry, were shown to experience toxicity from exposure to glyphosate herbicide at 10-100x below agricultural levels, with the herbicide formulations more toxic than glyphosate alone. Interestingly, a third microorganism tested was shown to be resistant to glyphosate. The authors conclude that glyphosate-based herbicides can significantly impact the diversity of food-interest microorganisms and that there is evidence for additional toxicity effects from adjuvants (134).

Glyphosate can have a negative impact on the growth of crops which should be tolerant to the herbicide. By acting as a 'chelating agent', glyphosate changes the availability of metal ions – forming compounds with ions such as magnesium, manganese, and nickel – or by displaying metal ions adsorbed onto soil particulates (118, 122). A study of glyphosate-tolerant soybean found that glyphosate treatment reduced nitrogen fixation in root nodules, as well as overall dry weight, the number of root nodules (essential for nitrogen fixation), and chlorophyll and nickel abundance in leaves (135). It is suggested that chlorophyll damage may occur via direct damage by AMPA, or because of chelation

of magnesium (a component of chlorophyll) or manganese (involved in photosynthesis). Root nodule formation may have been prevented by glyphosate chelation with nickel, reducing nickel availability for nitrogen fixation.

While glyphosate is the most used herbicide worldwide, there are other herbicides for which there are tolerant GM crops available. Tolerance to glufosinate is the second most common form of GM herbicide tolerance, yet there is significantly fewer ecotoxicity data available. Glufosinate has toxicity to aquatic organisms and soil microorganisms, and there is some suggestion of toxicity to predatory insects, mites and butterflies (136). It has been shown to disrupt reproduction and development in rats and rabbits (136), and is no longer approved for use within the European Union (137).

Two older herbicides, Dicamba and 2,4-D, have become increasingly common, along with glufosinate, as resistance to glyphosate increases (136). Dicamba and 2,4-D are synthetic analogues of the plant hormone auxin, and show toxicity to target plants 75 and 400 times greater than that of glyphosate, respectively. They are highly volatile, thus increasing the risk of interaction with non-target plants and other organisms, and have been found as having a carcinogenic activity (136).

There is evidence that the use of herbicide-tolerant crops has led to an overall reduction in herbicide use, and a reduction in the variety of herbicides used, largely as a result of the heavy dependence of glyphosate in conjunction with glyphosate-tolerant crop varieties (43). Similarly, the use of insect-resistant crops has led to a reduction in the use of insecticides, which can have severe non-target effects (43, 138, 139). The widespread adoption of such GM crops could be argued to have an overall benefit in the reduction of associated novel entities. This is likely to be more true in the case of insect-resistant crops, as the herbicide tolerance in crops is associated with a change in patterns of herbicide use, not just a simple reduction.

To conclude, certain associated novel entities present risks to biodiversity. Heavy reliance on herbicides such as glyphosate can result in changes to microbial community structure and could have severe negative effects on essential species including earthworms and bees. Increasing application rates of herbicides result in changing composition of weed communities, with knock-on impacts on insect communities and other animals reliant on these communities. The direct health effects of glyphosate exposure on animals including humans continue to be assessed, but current evidence is concerning. These risks are not unique to herbicide use on GM crops, however, heavy herbicide use is often inherent to GM crop cultivation, and thus can exacerbate these issues. Heavy reliance on pesticides for weed control is not sustainable; instead, a more holistic approach is required, which not only allows farmers control of weeds but can benefit biodiversity (122).

2.3 Evaluation of GM Crops with a Planetary Boundaries Framework

The simplest method for calculation of a planetary boundary for GM crop use is through associated novel entities, specifically through associated glyphosate. Glyphosate use is measurable – data is readily available for glyphosate application rates in the years 1994-2014. Data available on glyphosate degradation and persistence could be used to calculate the current levels of persistence based on current application rates, thus giving an estimation for current

environmental levels of glyphosate in a given area. Estimates of current environmental glyphosate concentrations would ideally be used in conjunction with monitoring glyphosate levels in a range of field situations, to validate and improve estimations. A simpler, although less ecologically relevant, measure of current glyphosate concentrations would be to estimate the highest concentrations after application, based on current guidelines for application rates.

It would also be necessary to define the point at which environmental glyphosate concentrations become unacceptable, which would be based on the currently available research. A lower limit might be the concentration at which sub-lethal impacts on keystone species are observed, and the upper limit the point at which lethality is observed in a wider variety of organisms, including keystone species, soil microbes, and aquatic organisms. Such limits would have to be based on currently available data but would be continually revised as new data is published.

In combination, the current environmental concentrations of glyphosate in GM crop areas, along with the limits set by non-target organism toxicity data would give a rudimentary local-scale planetary boundary for glyphosate-tolerant GM crops. This method is limited to glyphosate-tolerant crops, which although constitute a large proportion of cultivated GM crops, but still overlooks a large and potentially increasing proportion of available crops. The above method could be applied to other associated novel entities but relies on comprehensive data for application rates and amounts used by area, and an understanding of the ecotoxicological properties of each associated novel entity.

A similar approach could be taken for establishing a planetary boundary based on novel compounds, produced by the crop, where appropriate. Limits for environmental concentrations should be set according to toxicity effects observed in non-target species. Quantifying current levels is more complex; how the compound(s) is produced, how much is produced, where it accumulates, how it degrades, how long it can persist in soil or plant matter, whether it is exuded during the lifetime of the crop, and how much remains in plant matter left behind after the harvest, will all contribute to environmental levels. Given that all of these conditions vary greatly between GM crop varieties – even similar varieties – more experimental data will be needed to more fully understand these processes and their impacts on non-target organisms.

Establishing a planetary boundary must also consider other aspects of the risks associated with GM crop use, in particular the risks to contamination, biodiversity and weed dynamics resulting from transgene flow and volunteer escape. The understanding of contamination and transgene flow is, at present, too disparate to make general judgements beyond the acknowledgement that the risk is present. Current knowledge relating to the extent of transgene flow and other contamination events has depended on small-scale targeted monitoring events and in some circumstances luck, rather than widespread monitoring and sampling approach which would indicate the extent and frequency of such events.

Different crops pose different levels of risk, depending on a range of factors, from the propensity of seeds to spread from planting sites and during transportation, to pollen dispersal, and proximity to non-GM hybridising species and landraces. What is more, the mode of action by which transgene flow poses a risk to key Earth System processes is substantially different from those of associated novel entities and novel compounds. Risks posed to genetic diversity

and wider biodiversity are complex, dynamic and have many possible interactions with other processes. It is unlikely that we would be able to directly observe the impacts of transgene flow as they occur, particularly on shorter timescales. Setting limits for the transgene flow resulting from GM crop cultivation could, at least in part, be reliant on moral judgements on the ethics of human interventions in the genetics of domesticated and wild species.

3 Livestock

The livestock breeds familiar to us today were first domesticated 8000-10000 years ago. Modern selective breeding methods have been the mainstay of livestock breeding since the 1700s, until technological developments in the twentieth century introduced computing to assist breeding, and then later molecular and genomic breeding techniques. Molecular breeding involves the use of molecular biological techniques to identify genes and alleles conferring desirable or undesirable characteristics, to aid selection of individuals for breeding. Genomic breeding uses gene-editing techniques to specifically modify target genes or introduce genes to change the desired trait (140).

The use of GM in livestock breeding is still in the early stages, in comparison to GM crops. In the past, GM livestock breeding was limited by the difficulties in animal gene editing but has improved in recent years with the development of CRISPR Cas9. Genetic modifications have been used to increase muscle growth in swine, sheep, and goats. Swine have also been modified to produce higher levels of unsaturated fatty acids and healthier meat, and cattle to increase resistance to tuberculosis (140). There are many further suggested applications for the genetic modification in livestock; to improve product quality, reduce allergenic compounds, introduce or improve resistance to diseases, and to introduce new traits to a species (140, 141). The major benefit of gene editing over traditional breeding is the ability to make modifications that would be inaccessible to traditional selective breeding.

The commercialisation of GM livestock is still in its very early stages, partly due to the complexity of genetic modification in animals, and in part due to strict regulations. In 2015 the first approval for GM livestock in the USA was granted, for the AquAdvantage salmon. The developers, AquaBounty, modified Atlantic salmon to produce low but sustained levels of growth hormone, increasing the rate of growth and reaching market size two times faster than non-GM Atlantic salmon (142). As part of their approval by US regulators, AquaBounty was required to adhere to strict safety conditions for AquAdvantage farming, including land-based facilities to contain farmed salmon and prevent contamination of wild populations. AquAdvantage salmon farmed as food are sterilised as a further precaution against contamination of wild populations, although separate populations of fertile fish are maintained for breeding (142).

3.1 Risks of GM Salmon Aquaculture

The risks of GM aquaculture have been discussed at length by Le Curieux-Belfond *et al*, setting out the factors for consideration before wider adoption (141):

1. Assessment of environmental risk, in particular for biodiversity.

2. Assess the safety to the health of aquatic GMOs intended for animal feed or human consumption, in the short-, medium- and long-term.
3. Assess the social, cultural and economic impacts of commercialisation, in particular for individuals and small-scale aquaculture businesses.

The environmental risks of GM salmon aquaculture are similar to those of GM crops. GM salmon could escape containment and breed with wild populations, altering their biological function and ecological interactions. Escapes of farmed salmon have long caused problems for wild salmon stocks, damaging biodiversity and genetic diversity of local populations (141). As AquAdvantage salmon are currently farmed, the risks of escape and contamination are low, due to the strict containment procedures in place. If farmed AquAdvantage salmon do escape, they would be unable to breed with wild populations as they are sterile, although escapees from breeding populations would be able to interbreed with wild populations.

The safety of GM salmon for human consumption is likely to be an area of considerable controversy as AquAdvantage are commercialised. In 2015, the US FDA declared that the salmon were safe for human consumption (143, 144), stating that the nutritional profile of AquAdvantage salmon was comparable to that of non-GM farmed Atlantic salmon (144, 145). FDA approval also required consideration of direct effects of the novel genes – alterations in the gene products and associated proteins including allergens – and indirect effects arising from the novel genes – alterations in the compositions of edible tissues and associated allergenicity (144). The only significant difference identified between AquAdvantage salmon and non-GM salmon was elevated hormone levels, which were determined to not affect human health as they were within the range of those found in other food stuffs (144). FDA approval was met, at the time, with a backlash from consumer and campaign groups voicing concern over the extent of safety testing (143), indicating that even where regulatory requirements are met, the debate is far from over.

A general criticism of GMOs is the potential for off-target effects. These could arise as a result of modifications to non-target genes during the gene-editing process, or from modifying a target gene which has multiple functions or products. In the latter case, these should be identified early in the process of developing a GM livestock product, as they will likely have a significant effect on aspects of biological function. Indeed, target genes may be screened based on whether they have a single function or multiple functions, to simplify the development process. In the former case, again, off-target modifications should be identified during the development process, particularly where there is an obvious effect on biological function. Furthermore, newer gene-editing technologies such as CRISPR should be highly specific to the target gene, reducing the likelihood of off-target effects. Scientifically, the risk of off-target effects should be low, and the risk is therefore more likely tied to regulatory processes and business practices.

The potential for changes to aquaculture practices raises the question of what socioeconomic impacts may be brought about by the adoption of GM salmon. Some critics of AquaBounty argue that widespread adoption of the fast-growing AquAdvantage salmon would drive down the price of salmon to an extent that conventional aquaculture farmers would be forced to adopt the new technology, or result in small aquaculture companies going out of business (141).

AquAdvantage salmon farming is currently limited to three AquaBounty sites, and so does not occupy a large enough market share to have such impacts on aquaculture industries. The extent to which GM salmon, or GM livestock more generally will experience widespread adoption is, so far, unclear.

Questions have also been raised over the efficiency of salmon as a protein source; for 1kg of salmon flesh, 1.2-1.4kg of food pellets are required, in turn requiring 4-5kg of potentially food-grade fresh fish and shellfish (141). There are suggestions that future GM salmon could be developed to increase food efficiency, or a genetic modification could also be used to improve the nutritional quality of vegetarian salmon food. Regardless, the efficiency of salmon as a source of protein raises questions over whether GM salmon is likely to provide a solution to increasing global food requirements when other fish are more efficient for the conversion of calorie input to protein output. There is a concern that the increased rate of salmon production provided by GM salmon aquaculture would not benefit areas with the greatest deficits in protein production (141).

3.2 How GM Livestock is Regulated – deregulation for CRISPR edited livestock

Key to the development and commercialisation of GM livestock are the processes for governance and regulation for consumption, growth and import/export. GMO regulation varies considerably between jurisdictions, often involving multiple regulatory bodies and government departments. In some cases, regulations are dependent on the gene-editing method, particularly whether the organism contains novel (exogenous) DNA, in contrast to modifications to existing genes. In the USA, GM livestock products with no exogenous DNA are not regulated by the US Department of Agriculture, but are still subject to regulation by the Environmental Protection Association and are regulated by the same processes as drugs by the Food and Drug Administration. The EU, which has traditionally taken a more cautious approach to GMOs, regulates all GM foods under the same processes, regardless of the insertion of new DNA. In contrast, Argentina has embraced GM products and has taken the stance that GM products with no novel DNA insertion do not require regulation (140).

Before the AquaBounty AquAdvantage salmon application, the US FDA did not have the processes in place to evaluate and approve the application, contributing to an application process lasting almost 20 years (142). Strict regulations and extended application processes have received criticism from GM advocates as hampering the development of new GM products, particularly for smaller companies, for whom the high costs resulting from lengthy approval processes are prohibiting (142). Panjwani and Wilson (146) describe a cycle of public mistrust in GMOs: public mistrust in GMOs leads to tightening of regulations, driving up regulatory costs, and excluding small companies from GMO development. The resulting restriction of GMO development to large multinationals further increases public mistrust.

3.3 GM Livestock Contributions to a Novel Genetic Entities Planetary Boundary

Farming of GM livestock presents risks both to the genetic diversity of wild populations, through the potential for escaping and hybridisation, and to the wider environment through associated novel entities, such as effluent output

into water courses, food requirements and associated processes, energy requirements and efficiency, and antibiotics and other veterinary treatments.

As discussed in the context of GM crops, the impacts of transgene flow and contamination events are both difficult to establish and difficult to quantify. In the case of GM salmon, a hybridisation event has the potential to substantially change the growth rate of those carrying the transgene compared to those without, and thus change natural selection processes on such a population. It is well known that wild fish stocks are currently under pressure from overfishing, climate change, escapes and disease spread from conventional fish farming, pollution, and other anthropogenic factors (147-158). GM fish escapes and hybridisation has the potential to further exacerbate the current pressures. In such a scenario, the number of escape and contamination must be kept to zero.

Fortunately, the AquAdvantage salmon has an extremely low risk of escape and interbreeding with wild fish stocks, due to the extensive safety procedures AquaBounty has in place. The risks of transgene flow from GM fish farming would increase with widespread adoption, but would more likely arise as a result of negligence and undermining safety procedures, rather than purely as a factor of numbers. Monitoring is therefore an important aspect of maintaining the safety of GM fish farming, to reduce the likelihood of accidents and negligent practices.

Assessment of risk based on associated novel entities would be similar in the current scenario to conventional fish farming. Consideration would need to be given for all novel entities associated with fish farming (applicable also to other forms of GM livestock), but would rely on calculations of novel entity concentrations and limits set according to observed toxicity in non-target organisms.

It should also be noted that the GM livestock, particularly in the case GM fish farming, does have the potential to decrease the pressure on wild fish stocks, and thus improving population abundance and diversity in those situations. These benefits are not always realised, notably where fish farming increases the demand for fish oil and chum to feed predatory farmed species, thereby shifting fishing pressures to other fish stocks (154).

4 Biological Pest Control

The use of genetic modification to control pest insect populations dates back to the 1950s when radiation was used to sterilise large numbers of an agricultural insect pest before release. The sterile insects would then mate with the local population, but would not result in the production of offspring, decreasing the overall numbers of offspring produced. Continuous releases would, over time, reduce the population size. At the time, this method of pest control was termed the sterile insect technique, or ‘SIT’. The technique fell out of favour due to the unpredictability of radiation exposure in successfully sterilising individuals, but also substantially reduced the lifespan of irradiated individuals, thus reducing effectiveness (159).

An updated version of SIT was developed in the early 2000s for use in the mosquito *Aedes aegypti*, a vector of several viral diseases, including dengue fever and the zika virus. Oxitec, a company based in Oxfordshire, UK, has been

developing the 'release of insects carrying a dominant lethal', or 'RIDL' method since 2002 (159). The first of their commercialised RIDL *Ae aegypti*, OX513A, contains a lethal gene which can be suppressed when the mosquito consumes food containing the antibiotic tetracycline. Male mosquitoes carrying the lethal gene are released and mate with females of the target population. The offspring of these mosquitoes then inherit the lethal gene, which is not suppressed as there is no tetracycline, resulting in death during the larval stage. With continued releases, the population size is reduced over time. The transgene results in death, and as such, it should not be able to persist in the environment (160-162).

The RIDL technique was then developed further to specifically target females. In female-specific RIDL (fsRIDL), the lethal gene is only expressed in female larvae, causing death before reaching the adult stage. The male offspring still carry the lethal gene and go on to breed with future generations of wild females. As with the 1st generation RIDL technology, over time the population is diminished, but the gene is still self-limiting. The benefit of this 2nd generation fsRIDL is that fewer releases of male mosquitoes are required to achieve the same level of population suppression. Since it is only the females which blood-feed, male survival does not increase the spread of disease. Female-specific RIDL has been applied in *Ae aegypti* and developed in the Asian tiger mosquito (*Ae albopictus*) (163).

The first trials of the Oxitec OX513A mosquitoes were conducted in the Cayman Islands in 2009. They have subsequently been trialled in Brazil (2011), Panama (2014) and India (2017), with expanded trials in the Cayman Islands in 2014, and have received approval to begin trials in Florida in 2021 (159, 162). They have been adopted for use as part of mosquito control programmes in Brazil, since 2015 (159). The second generation of Oxitec *Ae aegypti* – OX5034 – were trialled in Brazil in 2019.

There is considerable interest in using RIDL technology in reducing the spread of malaria. Initially, *Ae aegypti* were targeted, due to the lack of treatments and vaccines against dengue fever (164). Malaria is carried by multiple mosquito species, making it more difficult to target with genetic techniques, and there are available treatments and prevention methods (164). Oxitec is now developing its 2nd generation RIDL method in two malaria-vectors *Anopheles albimanus* and *A stephensi* (161, 162).

Other genetic methods have also been considered for mosquito control, including modifying mosquitoes to produce infertility, to resist the disease-causing pathogen, or even to 'vaccinate' humans against the pathogen (165). Another technique in development has attempted to produce *Ae aegypti* mosquitoes which are paralysed after consuming a blood meal (166). Non-GM methods have been developed using the Wolbachia bacteria, either to sterilise released males (167), or to compete with and prevent the spread of viruses which cause disease in humans (168).

Oxitec also aims to use their approach for management of agricultural pests, and are developing RIDL in the fall armyworm (*Spodoptera frugiperda*), soybean looper (*Chrysodeixis includens*) and spotted wing Drosophila (*Drosophila suzukii*) (table 6). Glasshouse trials of the diamondback moth (*Plutella xylostella*) have been conducted in the UK and USA. Contained trials of the Mediterranean fruit fly, or Medfly (*Ceratitis capitata*), have been conducted in the UK,

Greece, Brazil, Morocco and Australia (161). Several strains of pink bollworm (*Pectinophora gossypiella*), a cotton pest with global distribution, have been developed and tested in caged field trials (169).

In addition to reducing the spread of insect-borne disease or crop destruction, a major benefit of the RIDL insect control technique is the reduction in the use of indiscriminate insecticides (170). RIDL mosquito control strategies are often cheaper than the use of chemical insecticide and habitat destruction strategies to control mosquito populations – in Florida the use of RIDL reduced mosquito control costs by around 50% (159).

Species	Pest Activity	Control Method
Aedes aegypti	Disease vector: dengue, zika, chikungunya, yellow fever	Oxitec Friendly™ (RIDL) (161)
Anopheles albimanus	Disease vector: malaria	
Anopheles stephensi	Disease vector: malaria	
***Spodoptera frugiperda* (Fall armyworm)**	Agricultural pest: general, maize	
***Chrysodeixis includens* (Soybean looper)**	Agricultural pest: soybean, cotton, maize, beans	
***Ceratitis capitata* (Mediterranean fruit fly)**	Agricultural pest: fruits, nuts, vegetables	
***Drosophila suzukii* (Spotted wing Drosophila**	Agricultural pest: soft and stone fruits	
***Plutella xylostella* (Diamondback moth)**	Agricultural pest: brassicas	
Aedes aegypti	Disease vector: dengue, zika, chikungunya, yellow fever	World Mosquito Program Wolbachia (168)

Aedes albopictus	Disease vector: chikungunya, zika, dirofilariasis	MosquitoMate ZAP (Wolbachia) (171)

Table 6: Summary of genetic and Wolbachia pest control strategies for mosquito species and agricultural pest species. Genetic pest control includes RIDL and fsRIDL systems.

4.1 Evaluation of the Risks

The risks to be considered with the use of GM insect control are similar to those of GM crops in agriculture; transgene flow and the potential for non-target effects, either by the release of novel compounds or through ecological interactions.

4.1.1 Transgene Flow

Theoretically, the risk of transgene escape from the OX513A mosquito should be low. There is no evidence that the transgene in OX513A can be transmitted to other organisms by any means, including ingestion and horizontal gene transfer (172, 173). OX513A RIDL mosquitoes are also 'self-limiting' – meaning that the transgene causes death in the insects which inherit it, and therefore limits the spread of the transgene in the wider population. The life expectancy of OX513A mosquitoes after release is an average of two days (174). Self-limiting and short life-expectancy should limit the risk of transgene flow, and of genetic contamination between introduced and local populations (160, 170). The fsRIDL method does not result in the death of all offspring, allowing the maintenance of transgenic males which go on to breed with future generations of wild females.

One of the main concerns raised with the RIDL method is with the role of tetracycline in suppressing the lethal gene. Tetracyclines are a group of antibiotics including tetracycline, doxycycline, oxytetracycline, and chlortetracycline. They are commonly used in human and veterinary medicine, and so may accumulate particularly in areas where sewage is treated. The concern is that the levels of tetracyclines in the environment at breeding sites may be high enough to suppress the lethal gene in RIDL mosquitoes, allowing survival of transgenic offspring. In a study of environmental tetracycline levels, no tetracyclines were found at breeding sites at high enough concentrations to suppress the RIDL transgenes. The authors concluded that levels of environmental tetracyclines were not high enough to increase the survival of RIDL mosquitoes (175).

Despite the presence of the lethal gene, in the lab, approximately 3% of RIDL *Ae aegypti* offspring survive. In field trials, there are also reports of surviving offspring which then interbred with the local population. After a trial in Brazil, between 5-60% of insects in the local target population had up to 13% of their genome originating from the Oxitec strain. Notably, this does not include the transgene (176).

The ability of transgenes to spread in the environment is partially dependent on the ability of the insects to disperse after release. Data from large-scale release and recapture studies found dispersal ability of modified *Ae aegypti* at field

sites in Brazil and Malaysia was an average of 52.8m and 58.0m, with a maximum of 220m (174, 177). Dispersal has also been raised as an issue for RIDL agricultural pests, particularly among organic farmers who have raised concerns that the spread of GM insects to organic fields would contaminate organic products and result in the loss of organic certification (178).

The use of non-lethal genes to control disease spread by mosquitoes, such as modification of mosquitoes to resist diseases, would require additional modification to ensure efficient spread of the transgene within the environment (165). The most common method discussed is that of gene drives – genetic mechanisms which increase the frequency at which the gene in question is inherited by the individual's offspring. In comparison to the RIDL methods, gene drive mechanisms are very much intended to spread within and between populations. Models have shown that more 'invasive' methods of genetic control, such as gene drives, would require fewer releases, but as they are more persistent would require greater monitoring and stewardship (160). It is unclear whether the increased costs of monitoring would outweigh the cost-saving of fewer releases. It is also arguable that the more invasive methods of genetic control have greater potential to cause environmental damage, through greater persistence and gene flow between populations.

4.1.2 Novel Compounds

The Oxitec RIDL system involves expression of two novel proteins; a fluorescent marker and the tTAV protein, which at high concentrations results in cell and organism death. A key assessment for the safety of RIDL control therefore the toxicity of these proteins to other organisms with which they come into contact in the environment.

In laboratory tests, no toxicity was observed in two predatory mosquitos (*Toxorhynchites*) fed exclusively on OX513A, compared to non-GM *Ae aegypti* (173). In research undertaken for trial approval in Florida, no significant difference was found in mortality, size or behaviour of guppy fish (*Poecilia reticulate*) fed with OX513A and non-GM adults (172). In a similar study, two predators and a parasitoid of the olive fruit fly (*Bactrocera oleae*) were studied for toxicity effects of RIDL olive fly consumption, with no significant adverse effects on survival observed (179). tTAV is unlikely to have any significant effect by ingestion, as there is no mechanism for the uptake of the protein into cells intact, and toxicity to RIDL larvae is specifically due to the high levels of expression overwhelming the normal cell gene expression machinery (173). The fluorescent marker has previously been used in many organisms with no adverse effects and has been evaluated for food safety by the US Food and Drug Administration (180).

These studies provide evidence for the safety of the RIDL system, although it is important to note that the number of organisms tested has been limited. This is likely due to the relative novelty of the RIDL pest control system, in comparison to the equivalent insect-resistant GM crop varieties, which have had a far longer commercial history and are cultivated widely. Should the use of RIDL insect control methods increase, more studies will likely be conducted, as part of the applications process for regulatory approval, or as part of ongoing monitoring, either by affiliated or external organisations.

4.1.3 Associated Novel Entities

A major benefit of the adoption of insect-resistant GM crops is the potential to reduce the application of insecticides, which can have severe effects on non-target organisms and the wider environment. Likewise, genetic methods of pest control reduce the need for such insecticides and are a highly specific, targeted method of pest control. GM IR crops still have the disadvantage of selecting for resistance traits in their target pest species, as do chemical pesticides. The use of a RIDL method of genetic pest control circumvents this issue and could be a solution to control of insect pests with resistance to multiple insecticides, including Bt toxins produced by GM IR crops.

The diamondback moth, a pest species of Brassicas (broccoli, cabbage, canola and cauliflower) is known to rapidly develop resistance, and currently has at least some level of resistance to over 90 pesticides (161, 181, 182). As discussed concerning herbicides, the development of resistance by the target pest often leads to increases in pesticide application volume or in the range of pesticides applied. RIDL diamondback moths, tested in glasshouse trials in the UK and US, containing an autocidal gene which results in female death during the larval stage, have been found to reduce the number of moths causing damage to crops, and over time reducing the size of a target population (178, 181). The use of RIDL could therefore be a significant contribution to the control of pests which are no longer effectively controlled by chemical methods, as well as preventing further development of insecticide resistance, by providing an alternative to chemical pesticides.

Similarly, in an environmental impact assessment before trials of Oxitec's OX513A mosquitos in Florida, it was determined that the current methods of mosquito control, largely reliant on chemical insecticides, had a greater negative impact on the wider environment than any potential risk from OX513A.

The use of genetic pest control methods to suppress insect populations has the potential to decrease reliance on chemical insecticides, which often have severe effects on non-target and beneficial insects. In this scenario, the use of a GMO could contribute to a reduction in certain novel entities, and improvements to biodiversity.

4.1.4 Ecological Interactions

A major consideration in the use of genetic methods of pest control is the role of the target insect in ecosystems. This is of particular concern where the target is a keystone species, where other organisms are dependent on the target insect e.g. as a food source, or where other invasive insects would be able to invade an eradicated insect's niche, ultimately resulting in ecological shifts. What is notable in the scientific literature of genetic technologies to control insect pests is the acknowledgement that the use of RIDL and fsRIDL to control a given pest must be examined on a case-by-case basis. Luke Alphey, one of the founders of Oxitec who also worked on the development of the RIDL mosquito, states that ecological impact will vary depending on the scenario; that suppression of recently-established invasive pests by RIDL is likely to have a considerably different outcome than in the area-of-origin, where the pest is native (170).

In the case of *Ae aegypti*, invasiveness can be considered key in assessing ecological impact. *Ae aegypti* is native to Africa but has spread to tropical and sub-tropical areas North, South and Central America, Asia and Oceania, pushing into more temperate areas as the climate warms (146, 161, 183-185). In these areas, the ecological impact of suppressing the *Ae aegypti* population, or potentially removing it completely, is less likely to have a significant ecological impact (170). The Environmental Impact Assessment for the OX513A trials in Florida found that as *Ae aegypti* is considered an invasive species, closely associated with human habitation, it is not an essential component of any local ecosystems. Any predators of *Ae aegypti* were considered to be generalist and opportunistic, rather than specific predators of that one species (172).

Of the other species currently under development for RIDL or fsRIDL, the malaria-vector *A stephensi*, as well as the agricultural pests the fall armyworm, Medfly, spotted wing Drosophila and diamondback moth have become invasive across various regions (161). In these scenarios, it could be argued that using a RIDL-based system would be an effective method to target and reduce invasive populations, with minimal impacts on the wider environment.

The issue becomes more complex when considering disease-vectors and pest species within their native range. To some extent, it could be argued that where human activity has increased the population of a given pest species, the use of a RIDL-based pest control method could still be reasonably used. Even so, within a native range, an organism is more likely to have a significant role in its ecosystem, through predator/prey interactions, through nutrient cycling, or through modification of some other resource. In these scenarios, the suppression or removal of a species may be counter-productive to long-term sustainability. The more appropriate method of genetic control for species within their native ranges could therefore be a non-lethal gene coupled with a gene drive, for example, a disease resistance gene.

As discussed above, transgene flow is inherent to gene drive mechanisms. In a hypothetical situation where a gene drive for disease resistance is introduced into one of the malaria-vector mosquito species within its native range, the assessment of any impact has to consider whether the spread of an introduced gene is acceptable. This will consist of assessing the same criteria as for the RIDL mosquitos, but with a particular focus on the potential risk associated with the gene, its direct and indirect effects on the biology of the target species, and any potential effect arising from toxicity or bioaccumulation of the gene product. The disease resistance mechanism must have as little impact on the biological functioning of the target species, such that there is no or minimal change to population dynamics as a result of transgene introduction. Similarly, there must be negligible toxicity of products arising from the transgene for direct predators, higher predators, parasites and parasitoids, decomposing organisms and the organisms which receive nutrients from decomposed material.

Ultimately, the extent to which the release of any GMO could negatively impact the environment is an essential component in evaluating the appropriateness of the method of pest or disease control. At present, there is a general requirement for assessment of any ecological impact before release, whether part of a field trial or for commercial

use. As part of trials of the OX513A *Ae aegypti* in Florida, USA, the FDA used the following parameters to assess the risk of GM mosquitoes becoming established and causing damage to the environment:

1. "The effect of the [transgene] on the fitness of the animal for the ecosystem into which it was released.
2. The ability of the animal to escape and disperse.
3. The stability and resiliency of the receiving environment."

Furthermore, the "overall risk is considered to be a product of all three, not the sum, and therefore if the risk of one aspect is deemed to be essentially zero, the overall concern is considered to be low" (172).

4.1.5 Transparency and Public Engagement

Regardless of the approach – RIDL or gene drive – there is still a conflict over the use of these organisms in pest control initiatives. One of the most prominent criticisms of Oxitec's RIDL mosquitos is centred on the transparency around the conducting of field trials. Some critics have claimed that the company took advantage of countries with weaker GMO regulation to push forward with field trials without sufficient review and public consultation (164). They have also been criticised for their engagement with stakeholders before, during and after the trial. Some have claimed that residents of the Cayman Islands were not made aware of the trial, or that information was not easily available, although Oxitec disputes this saying they engaged extensively with local communities throughout the trial process (146, 176, 186, 187). There was also controversy over a failure to engage with the international scientific community, with many claiming that the company did not publicise their trial until the year after it was conducted (186, 188).

Whether or not the lack of international engagement was intentional, many have suggested that any perception of secrecy surrounding such trials causes damage to the reputation of these technologies and stokes public mistrust (186). Key to the ethical implementation of GM mosquito trials is protecting the public and environment from harm, finding a balance between the benefits and risks, and collaborating with local communities to avoid exploitation and obtain consent from research subjects (165).

4.1.6 Global Health Ethics vs. Environmental Ethics

Nading (187) describes the conflict in ethical discussions of so-called 'global health GMOs' as bringing environmental ethic and bioethics into an 'uncomfortable proximity'. If one takes a 'health view' of global health GMOs, the critique from the 'environmentalist view' is that of toying with the balance of nature. In the reverse scenario, taking the 'environmentalist view', the response could be that of prioritising non-human health over human (187). The two are not always in conflict; in the case of RIDL suppression of invasive species with low ecological importance, genetic pest control could benefit both the environment and public health. In the case of ecologically important species, the conflict between health and environment are more obvious – the complete removal of a species has the potential to have drastic knock-on ecological effects. In the case of complete removal, the ethical question of whether humans should intentionally remove a species is raised.

A potential scenario involving a non-lethal gene coupled with a gene drive mechanism could be viewed as a way around the ethical dilemma of health vs. environment. Non-lethal disease resistance in this scenario would remove the issue of whether humanity should intentionally remove a 'pest' species, but raises additional questions over whether humanity should intentionally modify an entire species in such a way. If the use of a non-lethal gene drive is considered an unacceptable change to an aspect of genetic diversity, the issue reverts to this health vs. environment conflict.

It could be argued that human intervention in the genetic make-up of a wild species is comparable to other human activity, particularly that of domestication, which involves the intentional, but non-targeted genetic modification of wild or semi-wild animals by selective breeding. It is a common argument raised by advocates of GMOs, normally in the context of GM crops, but has a fundamental flaw in that it assumes all cases of such human interventions are fundamentally ethical. A similar argument is that many species become extinct, whether resulting directly from human-activity or in cases where the cause is less obvious, or unrelated to human activity and as such the removal of a single species would have a minimal effect in the grand scheme of extinction and evolution throughout natural history. It's unlikely that this argument would ever hold much weight – electing for a species extinction event is a dramatic and irreversible choice, and such human intervention in species survival is difficult to consider as ethical.

While such arguments may seem extreme, it is possible that soon it will be necessary to consider where such lines should be drawn. In some cases, this will involve conflict between cautious approaches to environmental interventions and potential benefits to human health. The answer is far from clear, but a clear and comprehensive understanding of the risks and benefits of GM pest control strategies, the alternative strategies, and inaction (146), will be essential to such an evaluation.

4.2 Do Genetic Pest Controls Represent a Planetary Boundary Issue?

Genetic pest controls have the potential for beneficial and adverse planetary boundary effects, the extent of which varies greatly depending on the insect and the genetic technique in question. Genetic pest control has the potential to reduce reliance on other novel entities, notably insecticides, and could have substantial global health or agricultural benefit. Transgene flow does pose a risk similar to that discussed in the context of GM crops and livestock, but there are some caveats to that risk.

While both insect-resistant GM crops and genetic pest controls can reduce reliance on insecticides, and have the potential for transgene flow, the risk of transgene flow occurring in crops is greater. Insects modified to control populations carry an intentionally detrimental gene, and therefore is not favoured by natural selection processes in the environment, unlike insect-resistant crops, which can gain a selective advantage over non-GM varieties. Furthermore, insect-resistant crops do not have limits to reproduction conferred by their transgene, whereas preventing reproduction is the fundamental mechanism by which genetic pest controls work. Thus, two GM approaches to control of agricultural pests have substantially different levels of risk with regards to transgene flow.

Climate change and other human activity is substantially altering, in some cases increasing, the range of many disease-vector and agricultural pest insects (184, 189-191). Without a substantial change in the current trends, conventional methods of insect control may be increasingly relied upon to combat these problems, increasing the levels of insecticides in the environment, contributing to a planetary boundary risk associated with those novel entities and knock-on effects in biodiversity and other Earth System processes. In such a scenario, there is the potential for genetic pest control to provide an alternative to widespread insecticide use or reliance on insect resistance crop traits. Genetic pest control could also represent an opportunity to directly target invasive species, where appropriate, in efforts to return ecosystems to a previous state.

In their current form, genetic pest control insects are unlikely to present a planetary boundary threat. Current patterns of use are of low risk to Earth System processes and could have a substantial benefit. This does not exclude the potential for future risk, and such risk would be highly dependent on each situation in which genetic pest control methods are applied, and the extent to which such methods are used. To prevent a planetary boundary threat developing, ongoing monitoring and careful consideration of the circumstances in which the technique is used would be necessary.

Calculating and quantifying planetary boundaries for.GM insects in genetic pest control is perhaps one of the most complex scenarios within the category of novel genetic entities. There is a necessity for considering both beneficial and adverse effects, including the risks posed by inaction (146). Determining a clear boundary, and quantifying its current status would likely be an impossible undertaking. Despite this, a planetary boundary-style framework could be used in the assessment of genetic pest control, to ensure that decisions are made based on an understanding of the potential range and direction of impacts posed by GM insects. An assessment of this type would need to consider the following:

a. The target species – its roles in the ecosystem and ongoing sustainable ecosystem function, as well as its invasiveness to the area in question.
b. The environment – how stable or resilient the environment receiving the transgene is to change.
c. The gene – its direct and indirect effects on the biology of the target species, and the potential effects of the gene product on other organisms and ecosystem processes.
d. The disease/pest – the extent to which a modification is likely to have an impact on the prevalence and spread of the target pathogen/pest, and the current impact of the pathogen/pest on individuals and communities.
e. The current approach – alternative approaches to tackling the disease vector/pest, and the level of risk in comparison to genetic methods of pest control.

Even in a situation where the use of genetic pest control was determined to be of great benefit and low risk, it should not be the sole approach for reducing the spread of disease. There is still a need for treatments, prevention strategies, and vaccinations; these should be the focus first and foremost. In the case of agricultural pest control, currently available alternative approaches, in the form of pesticides and insect-resistant crops, already pose a level of risk. While

genetic pest control could provide a benefit in reducing the dependence on these methods, it should not be viewed as the sole solution. It is still vital to explore other options to reduce the prevalence of agricultural pests, whether that is organic farming strategies, permaculture, or other approaches.

5 Other Applications for Genetic Editing

Gene editing is a hugely flexible scientific tool. New techniques for gene editing open up new possibilities for their application in research, medicine and industry. In this section, I will discuss examples of recent advancements and suggested uses of genetic modification.

5.1 Bioremediation

There have been suggestions that genetic modification could be used to produce organisms which aggregate and remove pollutants – another component of the novel entities planetary boundary category. GM microbes have been suggested for bioremediation of areas contaminated by heavy metals, petroleum-based pollutants and plastics, and organic compounds originating from agriculture, industry and military use (192). Genetic engineering of microbes for bioremediation could take any of several approaches; by improving the productivity of microbes already capable of metabolising the pollutant, by introducing new genes and pathways to a microorganism for the metabolism of a target pollutant, extending the range of pollutants a microorganism can metabolise, improving uptake of the pollutant into the cell, or improving the overall robustness of the cell to survive in harsher conditions (192).

GM microbes have the potential to spread quickly and extensively, contributing to the removal of pollutants from many environments across a wide area, and far more than more localised non-biological geoengineering. Taking a cautious stance over GMO use, the lack of control after release into the environment would be hugely concerning. However, some advocates for the use of GMOs in bioremediation argue that the reasonable level of risk posed by the release of novel GM microbes is far outweighed by the 'certain disaster' of inaction (193).

Another suggested use for GMOs in a conservation context is the potential for GMOs in detecting pollutants. Genetically modified zebrafish have been suggested for detection of pollutants in water, including oestrogen-like substances and hydrocarbons (141).

5.2 Medicine and Pharmaceuticals

5.2.1 Pharming – GMOs Used to Produce Pharmaceutical Products

The earliest applications of GMOs after their development in the 1970s was in the production of pharmaceuticals. In the early 1980s, the bacteria *Escherichia coli* was engineered to produce human insulin for diabetes treatments (194). GMOs are particularly well suited to the production of proteins for pharmaceutical use, as living cells can build the complex protein structures which would be difficult if not impossible to produce by chemical synthesis (195, 196).

At present, the range of pharmaceuticals produced by GMOs is extensive, with examples including the cancer drug α-interferon and surgery drug aprotin, as well as numerous vaccines and antibodies (195, 196). Biopharmaceuticals can be produced in cell cultures, such as the protein tPA which is a common treatment for stroke (197, 198). Whole tobacco plants were adopted early for the production of human proteins, including serum albumin (196, 199). The use of whole plants has been the subject of some controversy after cases of volunteer escape were discovered, as is often found in GM food crops (200). A subsequent shift in pharming away from whole crops grown outdoors, to indoor growing and cell cultures, aimed to reduce the risk of transgene escapes and recover the reputation of GMOs in pharmaceutical production (200).

Biopharmaceuticals can also be produced in animals. The first approvals of this GMO technology came in the mid-to-late-2000s, with approval in Europe and the USA for genetically modified goats producing the drug Antithrombin III in milk, for the treatment of antithromblin deficiency (195).

5.2.2 GMOs as Medical Treatments and Biomedicine

In addition to using GMOs to produce components of medicines, the advent of CRISPR gene editing has invigorated research into the possibility of using gene editing as a medical treatment (37).

One such example of GMO medical treatments is the use of modified viruses to treat persistent bacterial infections, in the form of biofilms (201). Biofilms are complex aggregates of structured microbial communities embedded in a polysaccharide matrix. Biofilm formation can occur in infections, in particular surrounding implanted medical devices, and are often associated with multi-drug resistance. Biofilms can also form on stainless steel surfaces, in industrial food processors and water treatment facilities. There is an interest in the possibility of using modified bacteriophages – viruses which specifically target bacteria – to prevent and remove biofilm formations, and as an alternative to or alongside antibiotics in medical and industrial applications (201). Modified bacteriophages have been developed which use the CRISPR gene editing system to remove resistance genes from antibiotic-resistant bacteria. Other modifications have been used to change the target bacterial species of a bacteriophage or to enable better disruption of biofilm formation (201). Bacteriophages are a promising option for tackling biofilms, as they are highly specific to a target bacteria species, where antibiotics are not. They are also self-limiting, only persisting and replicating where the host bacteria are present.

CRISPR has been used to 'correct' patient stem cells *in vitro*, which can then be transplanted into the patient, for example implanting functional epithelial cells into patients with cystic fibrosis (37, 202, 203). The approach has also been studied for application to genetic eye and blood diseases (204, 205). Recent work has investigated the use of CRISPR to target and correct key mutations in cancer cells, as well as developing modified immune cells for immunotherapies (18, 206).

5.3 Research

The use of genetic modification is essential to current scientific research. In genetics, genetic modification is used to disrupt the function of genes of interest, thus indicating their functions. It can also be used to examine the structure of DNA, how genes are expressed, control over gene expression, and examining the interactions between multiple genes and their protein products (18, 36, 37, 207, 208). These techniques are also applied to many other areas of research such as the study of diseases, including the genetic basis of dementia and other degenerative illnesses, cancers, and susceptibility to infections (209, 210). It is common in the study of genetics in disease to create models by genetic modification, ranging from fruit flies susceptible to Parkinson's disease to tumour-prone mice (211-217). GMO disease models can be used to study the development of the diseases in question, how they function, and in the development of new treatments (207, 218). The use of genetic tools can also be applied to understanding the normal function of biological processes, including early embryonic development, neurobiology, behaviour and processes such as wound healing and homeostasis (219-225).

Tagging of proteins with fluorescent markers can be used in the study of protein function, protein localisation within a cell or whole organism, and interactions between proteins in cell processes (226-228). Modifying organisms to produce proteins and other biological compounds, as discussed in 5.2 Medicine and Pharmaceuticals, also has applications in research, whether that is for the study of the protein itself – its structure, functions, characteristics and interactions – or as a method for producing proteins for use in other aspects of research, such as antibodies or fluorescent molecules (208, 227, 229-231).

It would be impossible to list all of how GMOs and genetic modification techniques are applied in scientific research; they are integral to much of the scientific progress from the past 20-30 years, particularly in the field of biological sciences and related disciplines.

5.4 Planetary Boundary Effects of Emerging Genetic Modification Applications and Technologies

The range of applications of gene-editing technology vary hugely, and new applications are constantly developing, particularly in research and medicine. When considering such applications in terms of a GMO planetary boundary, it is important to distinguish how these applications are fundamentally different from those discussed earlier, in that their contact with the environment should be minimal. GMOs used in research are confined to the laboratory, and according to regulations, must be destroyed after use. They are also often far weaker than their non-GM counterparts, reducing the risk for establishment in the environment. The risk can never be reduced to absolute zero, but an essential component of conducting research involving GMOs is adherence to risk assessments and regulations.

A similar argument could be made for medicinal products consisting of GMOs but becomes more complex when considering pharming. Industrial processes are sources of novel entities more generally, and thus it is not inconceivable that there is some risk of novel entities released in association with industrial cell-culture pharming. It should be noted that in such a scenario it is far more likely that novel entities resulting from pharming are associated novel entities,

rather than direct GMO risks. What is more, the use of biological processes to produce pharmaceutical products could have a lower overall planetary boundary impact, where the processes are more energy-efficient and involve fewer, less persistent or lower toxicity waste products than conventional pharmaceutical processes.

Pharming can also involve whole crops, which has been the subject of some controversy (200). Pharmaceutical-producing crops have the same issues like GM food crops, in terms of associated novel entities, novel compounds, transgene flow, contamination and volunteers. Production of pharmaceutical products is unlikely to increase fitness in escaped or hybrid plants, but contamination by volunteers can cause other problems where the plant produces pharmacologically active substances.

Bioremediation is substantially different again, involving intentional release of GMOs, and potentially widespread release. The intention for GMOs in bioremediation is to substantially alter the environment into which they are released – but to provide a benefit to Earth System processes. Bioremediation with GMOs has the potential to significantly reduce the impacts of other novel entities, such as plastics, heavy metals and chemical pollution, but must be done with care. It is essential to ensure that steps taken to restore natural ecosystems with GMOs will not result in further contamination or uncontrolled movement of the contaminants or their metabolites.

6 Looking to the Future: The next 30 years of GMOs

The current global trends of increasing population, increasing global temperature, and the increased effects of anthropogenic climate change present a huge and complex challenge for long-term sustainability and global development. Without a substantial change is those trends, there will be increasing pressures to provide enough food, from diminishing land, whilst also attempting to tackle the issues of inequality, disease, and reversing the current damage to the Earth System. How these issues might be tackled over the next 30 years is essential to predict the trends we might see in GMO use in that time.

The current trends in global GM crop use suggest that they will continue to be adopted in more countries, and planted over a larger area of cultivated land. At present, GM crop cultivation is dominated by herbicide tolerance and insect resistance traits. One change we may see over the next 30 years, assuming GM crops continue to be adopted, is a diversification in the type of genetic traits grown. As climate pressures increase, there might be increasing pressure on biotechnology corporations and agribusinesses to develop GM crops suitable for harsher growing conditions, so we might see an increase in crops with abiotic stress tolerance traits. It is possible also that there will be more small biotech companies addressing these needs, should larger corporations determine these GM crop varieties to be less profitable. Similar trends might be observed for disease resistance traits – where climate change and human activity increases the prevalence of pathogens – and in modifications to growth, yield and nutritional qualities, in an attempt to provide higher yields and more nutritious food over a smaller area.

It is also likely that over this time we will see an increase in stacked traits in GM crops. As resistance to broad-spectrum herbicides becomes more common, there will likely be a return to the use of many specific herbicides, and thus create

a demand for crops tolerant of multiple herbicides. There are already GM crops available with stacked herbicide tolerance traits, but if the current levels of HT crop use continue, then it is likely that a greater proportion of HT crops grown will have stacked traits against multiple herbicides, and increasingly against herbicides less commonly used at present.

As a result, we are likely to see more cases of volunteers, escape and transgene flow, and within that more cases of hybridisation – including hybridisation resulting in new combinations of stacked traits. This increases the likelihood that in the next 30 years, we will see new herbicide-tolerant weed varieties, and potentially new insect-resistant weed varieties as well.

Reliance on new GM crops is tempting, as it allows us to continue with our current systems rather than having to consider a major change to how we grow our food. It is essential that, even if we end up relying on some GM crops, we must not simply push back the problems of the current global food production systems to further in the future, or indeed simply replace current issues with new ones. Whether considering GMOs or conventional agriculture, it is clear that we must move towards long-term sustainability, and as such we must consider our reliance on GM crops within this goal.

While cultivation of GM crops has increased over the past 30 years, GM livestock has had a far slower adoption. This is in part due to the additional complexities involved in producing modified animals, but also due to the long process of regulatory approval, and a general reluctance towards their use. Nevertheless, there are developments in this area, and it is possible that over the next 30 years, more GM livestock will become available, particularly as CRISPR gene editing enables easier and cheaper development. At first, GM livestock production will likely be limited to the companies that developed them, as is currently the case for the AquAdvantage salmon, produced by the company AquaBounty. Should GM breeds of other livestock become available, then they could be adopted by conventional farmers.

What is difficult to predict is the appetite for GM livestock products – it is possible that, like GM crops, the idea of GM livestock will become more normalised in some areas, while other areas remain fervently against their use. It is also possible that there will be a general reluctance, if not outright refusal, to accept GM livestock products into global food systems. In either case, it may be that the investment required to develop GM livestock is simply too high for private companies to consider them worthwhile. It is also unclear whether the use of GM livestock would result in greater food production or increased efficiency of energy conversion (grams input versus grams output), or that GM livestock has any impact on the overall environmental impact of livestock agriculture.

Similar to GM livestock, it is as yet unclear whether genetic pest control will see widespread adoption. It has been slowly gaining traction and is being applied to an increasing number of species, however other techniques to control insect pests and diseases may be viewed as preferable – particularly in the case of disease vector species and the development of new vaccines and treatment options. There is also a possibility that similar, but non-genetic, methods will be viewed as preferable, in particular the use of the Wolbachia bacteria to control insect populations.

In medicine and research, gene editing will almost certainly continue to play an important role. The next 30 years are likely to include the development and adoption of new treatments involving genetically modified cells and products, and also the direct use of gene editing in medical treatment. There is also likely to be an expansion in the use of GMOs to produce pharmaceuticals and other products, in an attempt to reduce waste and increase the efficiency of industrial processes.

Pressures from climate change and anthropogenic Earth System change may push humans to reach for more and more elaborate solutions. In some cases, such innovations may be useful and necessary. This will not always be the case, and it is equally important to explore other options to reduce our impact. If we are determined to use GMOs to solve our problems, there needs to be a fundamental change, from perpetuating an intensification of global production systems to low impact, sustainable uses. It does not appear that current GMO use, or current trends, are meeting that objective.

7 Conclusions: A Planetary Boundary Definition for Novel Genetic Entities

Novel entities are defined as “new substances, new forms of existing substances, and modified life forms that have the potential for unwanted geophysical and/or biological effects” and are “of concern at the global level when these entities exhibit (i) persistence, (ii) mobility across scales with consequent widespread distributions, and (iii) potential impacts on vital Earth-System processes or subsystems” (2).

GMOs can act as novel entities, or novel genetic entities, according to this definition. Novel genetic entities show unwanted geophysical and/or biological effects, in the form of transgene flow, volunteer escape, changes to biodiversity, adverse ecological interactions, and associated novel entities. Novel genetic entities, their novel compounds and their associated novel entities can persist in the environment, long after their initial release. Living organisms inherently can spread, particularly where the transgene in question confers a fitness advantage. Novel genetic entities have the potential to impact vital Earth System processes, most notably those of biodiversity and climate change. In these ways, novel genetic entities can contribute to a planetary boundary.

Defining a planetary boundary for novel genetic entities is far from straightforward. The term ‘GMO’ encompasses and an incredibly broad range of organisms, techniques, and applications. Limits cannot be set based simply on the number of GMOs in environment, or the area they cover; a planetary boundary must take into consideration the interactions any given novel genetic entity might have, and take into account that some have a far higher potential for Earth System disruption than others. We must also take into account the potential for novel genetic entities to act beneficially, as well as adversely, and not assume that all GMOs will inherently result in negative consequences.

Despite the complexity, there are some indications of where we can start to build planetary boundary definitions and quantifications for some novel genetic entities. A more valid planetary boundary measure for novel genetic entities might be in the processes through which their impacts occur. These measures might include environmental concentrations of associated novel entities (herbicides, insecticides, other biocides, waste products), environmental

concentrations of novel compounds produced by the novel genetic entity (insecticides, growth factors), or a measure of hybridisation frequency (GM-conventional, GM-GM stacked hybridisation, interspecies hybridisation). Although these are ways in which planetary boundaries can be determined and quantified for novel genetic entities, they have limitations; not all GMOs are associated with another novel entity or interact adversely through their associated novel entities, adverse impacts arising from novel compounds may not have a linear relationship with quantity or concentration (as is the case for endocrine disruptors), and hybridisation risk varies hugely depending on the given situation. Such measures are simplistic and have the potential to be misleading, but in combination, they are reasonable a starting point for novel genetic entity planetary boundaries.

Except in the case of environmental glyphosate concentrations associated with herbicide-tolerant crops, there is not enough information available at present to fully define planetary boundaries for novel genetic entities. More research is needed to better understand the ecological interactions of novel genetic entities, their potential to interact with non-target organisms, to understand their efficiency for food production in comparison to conventional methods, their efficiency for industrial production, and how they are used in human systems. It is inevitable that when the interactions become more complex, it will be more difficult to understand how any given novel entity may act and interact.

We must also ask whether defining a planetary boundary is even appropriate for some or all GMOs, which can have contradictory beneficial and adverse impacts on different aspects of Earth System processes and may have complex, non-linear relationships with multiple associated novel entities and novel compounds. Should we instead consider another approach, one that does not attempt to quantify novel genetic entities? Perhaps we should take a more qualitative approach, that considers the range of novel genetic entity interactions, that can take account of emerging information, and that can account for contradictory benefits and risks in different areas. Such an approach would not be able to provide a clear target or limit for GMO use in these cases, but if combined with quantitative strategies, could help to greater inform our understanding of the current status of quantified planetary boundaries.

To assess the risks of genetically modified organisms within a planetary boundary framework, we must ask the following questions, considering them both quantitatively and qualitatively:

1. What is the direct risk of compounds produced by the novel genetic entity to all aspects of the wider environment?
2. What is the risk of movement of modified or inserted genes from the novel genetic entity into the wider environment, either as part of a whole organism, by hybridisation and introgression, or by horizontal gene transfer?
3. What is the risk of other novel entities or other key Earth System disruptive processes, occurring in association with the novel genetic entity?
4. To what extent does the novel genetic entity have the potential to reduce the risks of any of the above occurring as a result of alternative strategies currently or potentially in use for the same result?
5. Does the novel genetic entity promote long-term sustainability, or undermine it?

By taking a complex view, understanding that novel genetic entities have the potential to act and interact with many other organisms and processes, and over the short and long term, we can move towards a better approach for employing novel genetic entities in sustainable systems.

8 Glossary

Adjuvant – Any substance added to herbicide formulations to improve herbicide activity, application, or uptake (232).

Adsorption – The process of gas or liquid molecules forming physical or chemical bonds to the surface layer of a solid (233).

Allele – Different forms of a single gene e.g. a gene for flower petal colour may have two forms, one giving purple flowers, the other giving white flowers. Organisms may have multiple copies of a gene, which may be the same allele, or different alleles (234).

AMPA – Aminomethylphosphonic acid. The primary product from the breakdown of the herbicide glyphosate (235).

Biodiversity – Biological diversity. The variety of all forms of life, from genes to species, through to the broad scale of ecosystems (236).

Chelating – A process of molecules bonding to metal ions.

Community Composition – The number of species within a community, and their relative numbers (237).

CRISPR – Clustered Regularly Interspaced Short Tandem Repeats. CRISPR can refer to the section of DNA in bacteria which are involved in immune defence against viruses, upon which the gene-editing technology is based. It is also used as a shorthand for the gene-editing technique, based on CRISPR sequences and an enzyme, Cas9.

DNA – Deoxyribonucleic acid. The molecular material carrying genetic information, which self-replicates and is heritable.

Eukaryotes – A cell or organism consisting of cells with DNA contained in a distinct nucleus. Includes all organisms other than bacteria and archaea.

Exogenous DNA – DNA originating from outside the organism in question, that has been introduced into that organism (238).

Fitness – The extent to which an organism is well adapted to its environment, can survive, and ultimately reproduce and transmit genes (239).

Gene – A DNA sequence encoding one or more RNA or polypeptide (protein) products (240).

Genetic Diversity – The variety of genetic material within a population, species, group of species, or ecosystem. Can consist of variations in DNA mutations, alleles, genes or whole chromosomes.

Genome – The complete set of genes or total genetic material within an organism or cell.

Glyphosate – A synthetic chemical herbicide commonly used in agriculture. The active ingredient of glyphosate-based herbicides (GBH) is often a glyphosate salt.

Horizontal Gene Transfer – Exchange of genetic material between two contemporary organisms, as opposed to transfer of genetic material between parent and offspring organisms. For example, the transfer of circular DNA (plasmids) between bacteria. It is generally assumed to only occur between unicellular organisms and is more common between closely related species (241).

Hybrid – The offspring of two genetically distinct parents, of different breeds, varieties, species or genera. Hybrid offspring may be fertile, infertile, or semi-fertile (able to interbreed with one or both of the 'parent' species, but not with other hybrids).

Introgression – The process of a new allele or gene becoming 'fixed', or permanent, within a population, variety, breed, or species.

Keystone Species – A species within an ecosystem which are integral to the continuing function of that ecosystem in its current state, for example through interactions as predator or prey of one or numerous other species, or as 'ecosystem engineers' modifying the environment to provide nutrients, habitat or other services to other species.

Methanogenic/Methanogens – Single-cell Archaea which produce methane in low-oxygen conditions, common in moderate and extreme habitats (242).

Mutagenesis – The process of generating mutations in genetic material, either intentionally by gene-editing technologies, or unintentionally as a result of exposure to radiation, chemicals, or as mistakes in the process of DNA replication.

Prokaryotes – Single-cell organisms which do not contain DNA within a nucleus. Includes bacteria and archaea.

Resistance – The ability to continue function in the presence of an antagonist (e.g. disease, pesticide), either by disabling the antagonist or by use of alternative versions of the pathways targeted by the antagonist.

RNA – Ribonucleic acid. Molecular material carrying genetic information, with roles in expression and regulation of genes.

Shikimate Pathway – A metabolic pathway contributing to assembly of aromatic compounds, present in plants and some microbes. Glyphosate (herbicide) targets a component of the shikimate pathway, the EPSPS enzyme (243).

Stacked traits – Combining multiple GM traits in a single plant variety, the most common of which is stacked herbicide tolerance and insect resistance.

Tolerance – The ability to survive transient exposure to high concentrations of a chemical or pesticide (244).

Transfection – The process of introducing novel DNA into eukaryotic cells or organisms.

Transformation – The process of introducing novel DNA into prokaryotic cells.

Transgene – A gene which has been transferred from one organism to another, either naturally or by genetic engineering.

Trophic Levels – Hierarchical levels in the food chain.

Volunteers – GM crops which have grown from seed dropped by or dispersed from cultivated GM crops.

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