ADVISORY COMMITTEE ON RELEASES TO THE ENVIRONMENT

Report 2: Why a modern understanding of genomes demonstrates the need for a new regulatory system for GMOs.

Executive summary

Our understanding of genomes does not support a process-based approach to regulation. The continuing adoption of this approach has led to, and will increasingly lead to, problems. This includes problems with consistency, i.e. regulating organisms produced by some techniques and not others irrespective of their capacity to cause environmental harm.

Classifying the regulatory status of organisms produced by new techniques is becoming increasingly difficult, because the definition of a GMO in the Directive was drafted at a time when many of these techniques had not been conceived. Technology in molecular genetics is developing rapidly, to the extent that plants and other organisms can be modified in ways whereby the use of recombinant technologies leaves no detectable footprint. In some cases, this is because the organisms have not themselves been engineered using recombinant technology.

Our conclusion, that the EU's regulatory approach is not fit for purpose for organisms generated by new techniques, also applies to transgenic organisms produced by 'traditional' GM technology. Whilst it is clear that these will be captured by the GMO legislation, the potential for inconsistency is inherent because they may be phenotypically identical to organisms that are not regulated.

We have highlighted the problems associated with a process-based regulatory system, and suggest there are benefits of a different system that can take account of the novelty of the final product. Such a system is independent of newly arising and currently unforeseen technological developments, and focuses on potential risks associated with phenotype. This approach has many advantages over the existing regulations and should be considered as part of any proposal to improve or replace them.

Introduction

The Advisory Committee on Releases into the Environment (ACRE) is an independent advisory committee composed of leading scientists and technical experts. Our main function is to give statutory advice to UK ministers and ministers in

the Devolved Administrations on the risks to human health and the environment posed by the release and marketing of genetically modified organisms (GMOs).

This report is the second of three that consider the regulatory framework[1] in which we operate. Report 1[2], entitled '*Towards an evidence-based regulatory system for GMOs'*, identifies fundamental problems with the current legislation and proposes a different approach. Report 3[3], '*Towards a more effective approach to environmental risk assessment under current GMO legislation'*, identifies problems with the implementation of the current legislation that could be addressed without altering the legislation, and is focused on the environmental risk assessment of GM crops. In this report we consider how fit-for-purpose the current GMO definitions and regulations are for organisms generated by newly emerging modification techniques, and in the light of current knowledge of genome structure and epigenetics.

The first iteration of the EU Directive that controls the deliberate release of genetically modified organisms (GMOs) into the environment was adopted in 1990. This, along, with the directive controlling the contained use of genetically modified micro-organisms, established the EU's approach to defining GMOs. This approach is to define an organism based on how it was made and the nature of the resulting alterations to its genetic material. However, a number of reports, including the last review of the current 2001/18 Directive (1), have highlighted concerns about the clarity of the definition of a GMO when applying it to organisms produced by particular new methodologies, which we describe here as new techniques (NTs). Reports that have considered the issue in more detail include that from an EU Commission Working Group on 'New Techniques' (i.e. NTs, 2) and a series of papers by COGEM (3-5). A report from the Joint Research Centre also provides useful background on the NTs (6).

To address the issue further, in 2012, UK regulators asked ACRE to provide a scientific view on how the definition of a GMO in the legislation should be applied to plants produced by NTs. We provided detailed advice on the most credible scientific interpretation of each NT (7). This report led us to conclude that the definition of a GMO is unclear and, by extension therefore, the regulation of products generated using NTs will also be characterized by uncertainty.

The definition of a GMO in the Directive is based upon the techniques and knowledge of genomes available in 1990, when it was first implemented. A wealth of data generated in genomic studies since then highlights the weaknesses in the definition of a GMO, especially because it defines a GMO according to the method by which it was made. We discuss this evidence and its relevance to the regulation of GMOs in this paper.

[1] Legislation controlling the Deliberate release of GMOs into the Environment.
[2] http://www.defra.gov.uk/acre/files/Report-1.pdf
[3] http://www.defra.gov.uk/acre/files/Report-3.pdf

Whilst it is clear that the Directive's definition of a GMO does apply to most globally marketed transgenic organisms, evidence from the assessment and widespread cultivation of GM crops since 1990[4] combines to support our wider conclusion that it is not appropriate to regulate organisms produced by NTs.

There are many consequences that result from the adoption of a definition that has different interpretations and which is no longer founded on sound scientific principles. In this paper we present the arguments for a regulatory approach that is more consistent across the range of new products, based on the novel characteristics of the organisms produced. We also demonstrate how the lack of clarity about the regulatory status of a number of NTs can have an important impact on innovation and economic growth in crop biotechnology.

The Definition of a GMO

Article 2 of the EU's Directive on the Deliberate Release into the Environment of Genetically Modified Organisms (GMOs) (Directive 2001/18/EC) defines a GMO as:

> ***"...an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination."***

Within the terms of this definition, the Directive provides examples of techniques that either do or do not lead to genetic modification. These examples are included in annexes (these are provided in Annex 1 to this paper) and their inclusion highlights the governing principle of the Directive: that it is the technique used to alter an organism's genetic material that determines whether or not it is captured by the legislation – the term 'process-based' has been used to describe this approach.

Clearly, if the process by which an organism has been developed is captured by these annexes, the regulatory status of that organism (i.e. whether it is a GMO or not) is explicit. However, since NTs are not listed in the annexes, the decision as to whether plants produced by many NTs are defined as GMOs hinges on whether the Article 2 definition applies. There are different views on how Article 2 should be interpreted. Whilst the use of NTs leads to artificially generated changes to the organism's genome, there are differing opinions as to whether this is relevant, if these changes could have occurred naturally by breeding, mutation, recombination and selection. In our report on NTs (7), we concluded that Article 2 did not apply if changes in the

[4] The global area of GM crops has risen steadily to around 170 m hectares in 2012 (or about 7 times the land area of the UK). In that year, for the first time, the developing world grew more biotech crops (52%) than the developed world (48%) (http://www.isaaa.org/). For both soybean and cotton, 81% of global planted areas are now sown with GM varieties

genetic material of the organism could have occurred naturally. We also noted that it would be very difficult to enforce the legislation if Article 2 were interpreted differently. This is because many of the organisms produced by these NTs would be indistinguishable from those produced by conventional (non-GM) techniques.

Knowledge of genome plasticity

To determine whether the change to the genetic material could have occurred naturally, it is necessary to understand the level and types of genetic variation that can occur within and between organisms. Since 1990, when Article 2 was first adopted, our understanding of this baseline of variation has increased significantly through extensive studies of genomes. The result is that there is now a large body of scientific evidence demonstrating that within an individual and between individuals of the same species, the genetic material (genotype) can exist in naturally variant forms, both in terms of the sequence of the DNA and also in terms of its chemical structure (so-called epigenetic[5] modifications). In other words there is a high degree of natural plasticity and variability between genomes and epigenomes of individuals of the same species as well as within any single individual.

In 2012, Weber et al. (8) presented a detailed analysis of how genome plasticity is relevant to the issue of safety in GM crops and provided a number of examples of naturally occurring mechanisms that give rise to genome variation and plasticity.

This evidence helps to establish that genomes or genotypes exist in highly plastic and variable states between and within individuals; in some instances such variants may have virtually identical biochemical and physical characteristics (phenotypes). It is also axiomatic that genetic and epigenetic changes can both result in altered phenotypes, and indeed this variation is the basis of evolution by natural selection.

Additional evidence of genome plasticity in plants comes from recent studies involving new sequencing technologies used to explore the diversity that exists in the genomes and epigenomes of plants (9-16). The results of these analyses highlight the variation in DNA sequence, the differing copy numbers of particular sequences (17) and the variation in the methylation pattern of the DNA of individual plants within a species. For example, in a study of heritable epigenetic polymorphisms using 10 lines derived from a common ancestor 30 generations previously, it was shown that epimutations (stable epigenetic modifications affecting phenotype, i.e. outward characteristics) at individual loci were easily detected and that approximately 30,000 cytosines in each strain were differentially methylated (18).

[5] Epigenetic refers to alterations in the genetic material which lead to changes in gene function *without* a change in the nucleotide sequence of the DNA. The modifications are often but not always heritable.

Such variation underlies the traditional processes of plant and animal breeding and it should also be noted that such plasticity in genomes (19) and epigenomes is not restricted to these organisms, but extends from bacteria (20) to humans.

For example, the Encyclopaedia Of DNA Elements (ENCODE) project has revealed that much of the human genome gives rise to RNA molecules that do not code for proteins, but which can regulate gene expression and therefore alter the phenotype, such as through epigenetic mechanisms. This newly recognised role for what had previously been considered 'junk DNA' may represent a further source of genetic and epigenetic variation between individuals of the same species (21).

Such plasticity is being studied in systems where greatly different phenotypes are under epigenetic control. For example, it has been shown recently that the caste structure of ant societies, which is characterised by large numbers of individuals displaying different morphologies and behaviour, is largely controlled by epigenetic alterations that become functional during embryo development (22). This example illustrates how high levels of natural variation in epigenetic features of genomes occur and can influence phenotype.

The forms of genome plasticity described above have evolved to different extents in different orders of life forms (19), and although there are some theoretical limits to the boundaries of this variation (23), it is clear that the introduction of one or a few genes into an existing variety of plant represents a very small alteration in comparison to that which exists across the natural spectrum.

When Article 2 was written, the genomes of organisms were considered to be relatively uniform and stable (with global variation in genomic sequence and epigenetic heterogeneity uncharacterised). Therefore Article 2 would have been expected to capture the expected range of artificially induced alterations to an organism’s genetic material. Now that the extent of natural variation is more fully understood, identifying alterations that do not occur naturally by mating and/or natural recombination is more challenging and this approach to defining organisms is highly questionable. This is because within an individual and between individuals of the same species, the genetic material can exist in naturally altered forms whilst the phenotype remains unchanged. Equally, a single nucleotide change can result in a significant phenotype change.

The current regulations argue that it is the technique used to make the alteration to an organism’s genetic material that is relevant in determining whether the organism should be as a GMO (irrespective of the alteration introduced). This is based on a concern that there will be undesirable, unintended changes to the organism’s characteristics associated with the use of the technique. However, unintended effects are not specific to NTs or to recombinant DNA technologies; conventional breeding programmes select for organisms with desired characteristics and discard the majority of organisms produced, including those with unintended and undesirable features. Our

improved understanding of genomes puts these changes into the context of the underlying genetic and epigenetic variation present in these species.

In conclusion, the adoption of a regulatory approach based on how the genome of an organism is modified (rather than on the novel characteristic(s) that this change confers) is not reconcilable with the knowledge that we now have about the plasticity of genomes. This has a number of consequences in addition to the difficulty in interpreting the definition in a scientifically credible manner.

1. Problems associated with defining and regulating organisms based on how they were modified.

Problems of definition and regulation are loosely categorised here based on the type of problem presented. In some cases the status of NTs serves to illustrate particular issues. NTs include cisgenesis, site directed mutagenesis and RNA-dependent DNA methylation. Annex 2 provides a summary description of each technique.

It is inevitable that the regulatory classification of organisms based on their genotype and on the technique(s) used to produce them will lead to inconsistency. We highlighted this in our 2007 report '*Managing the Footprint of Agriculture: Towards a Comparative Assessment of Risks and Benefits for Novel Agricultural Systems'* (24) when discussing herbicide tolerant (HT) plants. We noted in this report that the environmental impact of HT plants produced using recombinant DNA technologies would be assessed and controlled, whereas plants generated via traditional mutagenic techniques would not. We also discuss this disparity in Report 1.

This inconsistency is likely to become more evident when considering organisms produced by NTs. Examples include cisgenic organisms and organisms produced using novel mutagenic techniques. In the case of the latter, it is possible that organisms that not only exhibit the same trait but which are made using very similar techniques will have a different regulatory status.

This raises another problem with the current regulatory approach, which is that definitions based on genotype changes and the use of particular techniques are open to interpretation and are difficult to future-proof.

For example, plants developed using traditional forms of mutagenesis (e.g. using chemicals or ionising radiation) are exempt from GMO regulation because 'mutagenesis' is listed in Annex 1B of Directive 2001/18/EC (see Annex 1). However, the same trait in the same organism caused by the same sequence alteration but using a different mutagenic process (such as zinc finger nucleases, transcription activator-like effector nucleases (TALENS) or oligonucleotides) may need to be regulated. This will depend how the EU Commission and regulators in the 28 EU

member states interpret the inclusion of ‘mutagenesis’ in Annex 1B. Their views on whether more modern methods of mutagenesis involve the use of ‘recombinant DNA techniques’ is also a pertinent issue in this debate. It is likely that there will be a range of conclusions reflecting those set out in the EU Commission’s working group report on NTs (2).

Other NTs may include a technique that is captured by the definition of a GMO in one step of their development process, although *the organism produced at the end of that process will not contain any inserted or recombinant DNA*. An example is reverse breeding. As the *process* requires the use of a GM intermediate, some regulators are likely to argue that the resultant organism is captured by Directive 2001/18/EC. Others may argue that, because the alteration to the final product could have occurred naturally or through conventional breeding, Article 2, and therefore Directive 2001/18/EC does not apply.

RNA-dependent DNA methylation (RdDM) induces an epigenetic change to the organism which produces the (heritable) desired trait. The sequence of the organism’s DNA is not altered, but the chemical structure of the DNA is modified. However, this alteration (usually methylation of a specific DNA base) results from a naturally occurring biochemical process inside the cell, making it particularly difficult to interpret the definition in this case.

Clearly any lack of consistency between different authorities on the interpretation of the status of organisms produced by different methods is undesirable. It raises the possibility that two different organisms, each with identical DNA sequences (genotype) and identical biochemical and physical characteristics (phenotype), may be regulated in one case but not the other. Added to this is the possibility that different EU member states may differ in their views. It is also likely that different regulatory systems outside of the EU will come to different conclusions.

In addition, NTs will continue to be developed through technological progress and scientific breakthroughs. An option for managing this might be to agree an EU position on each new technique as it arises. This will be challenging for the reasons described above. Consequently, it is unlikely to provide the regulatory certainty that is required to support innovation. It also perpetuates a system that is not scientifically defensible.

2 Innovation and trade is stifled

Different interpretations and inconsistencies surrounding the status of organisms are likely to have a negative impact on economic growth and development in the EU. For example, an applicant in the US recently received advice from the regulatory authorities that a biolistics-generated ‘GM’ grapevine containing grapevine anthocyanin regulatory genes ‘does not require regulation under the Plant Protection

Act'. As a result this product should be able to generate potential commercial benefits very quickly, contributing to the economic development of the sector in US.

In the EU, it is currently not clear whether this product, as a cisgenic GM organism, would need to be regulated under Directive 2001/18/EC. In the absence of such clarity, vine growers in the EU are unlikely to be willing to negotiate access to this novel product, and therefore will not gain any commercial benefit. For the same reason, EU businesses would be unlikely to invest in developing similar traits. Consequently, innovation and economic development in the EU is negatively affected by current uncertainty.

The high cost of processing an application through the EU regulatory system means that, currently, only those crop/trait combinations that have a high probability of realising their economic potential are being developed. These include traits such as Bt-mediated insect resistance and herbicide tolerance in crops such as maize. Other traits, likely to be associated with smaller profit margins, but offering a variety of solutions for crop protection, sustainable agriculture and delivering public benefits, are unlikely to be developed in the EU because the high regulatory costs coupled with the unsatisfactory regulatory regime make the financial risk too high. We conclude that the EU regulatory system is a barrier to economic growth and development.

The financial "rewards" for developing methods and products that are not covered by the regulatory framework are still potentially very large. Companies therefore invest significant resources exploring routes for avoiding 'capture' by the regulation. This might involve using NTs in the hope that the resulting product will fall outside of the regulation. Even though there remains a lack of clarity regarding the status of these organisms, such approaches make economic sense to the industry because of the slow, costly and frustratingly unclear GMO regulatory system.

This situation raises conflicts of ideology that span philosophy and economics regarding the application of GM technology for environmental benefits. For example, it is our view that in a climate of ever increasing global environmental pressure, diminishing resources and economic stagnation, *not* to explore the most sustainable and scientific way of solving a problem should be considered both perverse and unethical. A rethink of the regulatory framework would address such wide-reaching conclusions.

The need for a phenotype–based regulation

Whilst there is potential for EU member states to agree on the legal status of NTs, it will not be possible to solve the inherent problems that are the consequence of adopting a 'process-based' approach to regulation. In particular, there is potential for organisms with the same characteristics to be treated differently because of the focus on how they were produced and for techniques to be captured or excluded, when it is

not clear how the technology will develop in the future (in different types of organisms). Therefore, although short term solutions may be attractive, they perpetuate an approach that is not consistent with the scientific evidence and that will continue to cause problems in the future.

An alternative regulatory approach is to classify organisms based on the novelty of their characteristics – a so-called 'phenotype-based' approach, as defined in Report 1. We recommend that regulators consider replacing the current legislation with a framework that adopts this approach. We will not discuss the details of such a framework in this paper. However, we included an outline of a 'phenotype-based' approach for assessing novel agricultural products and processes in our report 'Managing the Footprint of Agriculture *Systems'* (24 - see also Report 1). It is also notable that Canada operates an effective regulatory system that captures novel organisms based on their phenotypic characteristics rather than the process that was used to produce them.

Moving to a phenotype-based approach would be more consistent with scientific understanding and would provide an opportunity to address problems inherent to the current process-based system. In particular, it should offer a more consistent and flexible approach to regulation.

A phenotype-based approach would enable classification and risk management of new organisms and products based on the novelty of their observable characteristics (i.e. phenotype) compared to existing organisms and products. Over time, certain products will no longer be considered novel and could be deregulated based on a safe history of use. This would reduce the regulatory and administrative burden.

Furthermore, a phenotype-based approach recognises that phenotypic changes can be significant, whether produced by current GM technology, NTs or conventional plant breeding. It also takes into account the fact that unintended consequences can potentially occur, regardless of the method used to alter the genetic material. The risks associated with a new product would be assessed according to the properties of the product rather than on the way the product was made, ensuring the safety of all new products.

Acknowledgement: ACRE thanks Professor Phil Mullineaux for his constructive comments in reviewing this report.

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Annex 1

Directive 2001/18/EC

Article 2

"organism" means any biological entity capable of replication or of transferring genetic material;

"genetically modified organism (GMO)" means an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination.

Within the terms of this definition:

- genetic modification occurs at least through the use of the techniques listed in Annex I A, Part 1; the techniques listed in Annex I A, Part 2, are not considered to result in genetic modification.

Article 3.1

This Directive shall not apply to organisms obtained through the techniques of genetic modification listed in Annex I B.

Annex I A

Techniques referred to in Article 2(2)

Part 1

Techniques of genetic modification referred to in Article 2(2)(a) are inter alia (among others):

- Recombinant nucleic acid techniques involving the formation of new combinations of genetic material by the insertion of nucleic acid molecules produced by whatever means outside an organism, into any virus, bacterial plasmid or other vector system and their incorporation into a host organism in which they do not naturally occur but in which they are capable of continued propagation;
- Techniques involving the direct introduction into an organism of heritable material prepared outside the organism including micro-injection, macro-injection and micro-encapsulation;

(3) Cell fusion (including protoplast fusion) or hybridization techniques where live cells with new combinations of heritable genetic material are formed through the fusion of two or more cells by means of methods that do not occur naturally.

Annex I A

Techniques referred to in Article 2(2)

Part 2

Techniques referred to in Article 2(2)(b) which are not considered to result in genetic modification, on condition that they do not involve the use of recombinant nucleic acid molecules or genetically modified organisms made by techniques/methods other than those excluded by Annex I B:

- in vitro fertilization;
- natural processes such as: conjugation, transduction, transformation;
- polyploidy induction.

Annex I B

Techniques referred to in Article 3

Techniques/methods of genetic modification yielding organisms to be excluded from the Directive, on the condition that they do not involve the use of recombinant nucleic acid molecules or genetically modified organisms other than those produced by one or more of the techniques/methods listed below are:

(1) mutagenesis,

(2) cell fusion (including protoplast fusion) of plant cells of organisms which can exchange genetic material through traditional breeding methods.

Annex 2- New techniques

New technique	Summary description
Cisgenesis /intragenesis	Cisgenesis generally involves the insertion of a complete gene sequence from the same or a closely related species (which is sexually compatible) by conventional genetic modification (cf Annex 1A). The inserted DNA sequence (cisgene) may already be present naturally in the genome. Intragenesis is essentially the same as cisgenesis but the introduced DNA has been 'reorganised' before insertion.
Site directed mutagenesis	*Oligo-directed mutagenesis* Involves exposing plant cells in culture to short fragments of DNA ("oligos", typically 20-100bp) which act as a mutagen inducing predictable changes (often very small 1 or 2 bp) to the DNA sequence. Because changes are 'targeted' it is an effective way to produce a new trait. The oligo DNA molecule is present only transiently and the change in host DNA sequence (which is stable and inherited) results from the activation of the host cell DNA repair mechanism. (In non-plant systems ODM can be used to generate larger sequence changes through homologous recombination) *Zinc finger nuclease (ZFN) directed mutagenesis* ZFNs are proteins that induce a break in the DNA of a plant cell at predefined genetic location/sequence. When the DNA strand is repaired (using the cell's own machinery) a change in the DNA sequence (mutation) may be induced (for example addition or deletion of a bp) and selected for. The ZFN protein is generally delivered to the cell using a) conventional genetic modification b) transient expression from plasmids or viral vectors. With the former, the transgene can be removed from the final product using backcrossing. With the latter, expression is transient and the final selected product will not contain a ZFN gene. ZFN is similar to ODM because the proteins act as a mutagen inducing predictable changes (often very small - 1 or 2 bp) to the DNA sequence.
RNA dependent DNA methylation (RdDM)	RdDM can generate stable changes to gene expression and plant phenotype, but there is no alteration to the DNA sequence of the host. Rather a change in the *structure* of the DNA molecule gives rise to the new trait/altered characteristic. This type of alteration is known as epigenetic modification. No new genes are present in the final product although cell culture and molecular biological techniques may have been used together with transgenic intermediates in the early stages of development.
Reverse breeding	The products of reverse breeding generally will not contain any new / inserted DNA and could be produced, albeit much more slowly, using conventional breeding techniques. Briefly, an elite heterozygous crop plant is genetically modified with a gene that causes a reduction in the number of chromosome cross-over events during meiosis. Gametes from these plants (half of which do not contain the transgene) can then be cultured to give rise to homozygous plants (double haploids) which are then crossed together to 'reconstitute' the heterozygous genome of the elite parent crop. This technique is very valuable to the plant breeding industry because the resulting progeny demonstrate characteristically enhanced vigour.