

Products of new genetic modification techniques should be strictly regulated as GMOs¹

New genetic modification techniques (NGMTs) are increasingly being developed and applied to generate new varieties of food crops and livestock animals. They are also being used for other purposes, such as to develop gene drives². They include – but are not restricted to – CRISPR-Cas/Cpf, TALENs, zinc finger nucleases, oligonucleotide directed mutagenesis, cisgenesis, transgrafting, and RNA-dependent DNA methylation. These techniques are sometimes referred to as “new (plant) breeding techniques” (NBTs or NPBTs)³. Some of them are also referred to as “genome editing” or “gene editing” techniques (CRISPR-Cas/Cpf, TALENs, zinc finger nucleases, oligonucleotide directed mutagenesis). These genome altering tools are also being used to expedite developments in synthetic biology, as one of the aims of these developments is to engineer novel biochemical pathways, and thus characteristics, into organisms ranging from viruses, bacteria and plants to animals⁴. While in medicine these methods are recognized as important tools that produce unprecedented genetic modifications, advocates in other disciplines seem to suggest that a different standard should be applied to their application in other fields. Such is the case in what we term here environmental applications, including agriculture as well as the management of a diversity of other ecological situations, e.g. insect-vectored epidemics, weed-control, and many others. The signatories below assert that products of NGMTs should be strictly regulated as GMOs.

Proponents say that NGMTs should not be regulated as GMOs

Advocates of NGMT use in environmental applications claim that viruses, microbes, plants or animals produced via these techniques are not genetically

¹ GMO = genetically modified organism

² Gene drives are genetic modifications that are designed to rapidly spread a trait or a handicap through populations or entire species of animals (e.g. mice, mosquitoes, flies) or plants (e.g. ‘weeds’, invasive species). They are advocated for various reasons, including efforts to eradicate whole populations of pests or carriers of human or animal diseases (e.g. insects such as mosquitoes that carry human malaria pathogens, or flies that eat the cherry fruit in orchards).

³ Lusser, M., Parisi, C., Plan, D., Rodríguez-Cerezo, E. (2011) New plant breeding techniques: State-of-the-art and prospects for commercial development. JRC Scientific and Technical Reports, EUR 24760 EN. Publications Office of the European Union (Luxembourg), EUR — Scientific and Technical Research series, doi:10.2791/54761, <http://ftp.jrc.es/EURdoc/JRC63971.pdf>

⁴ PLOS Collections (2017). Synthetic biology: Genome editing. <http://collections.plos.org/synbio-genome-editing>

modified organisms (GMOs) *per se* and should not be regulated as such. It has been claimed, for instance, that small⁵ base unit changes and altered function via epigenetic manipulation in one or more DNA sequences should not be regulated in agriculture, irrespective of consequences, based on the notion that mutations happen in nature. The proponents of NGMTs are now lobbying strongly to prevent regulation of the products of these techniques altogether, or at least to grant them “light-touch, product-based” regulated status. These efforts aim at reducing or avoiding safety evaluation prior to release as well as post-release labelling or monitoring, in order to allow swift marketing. “Product-based” (sometimes called “trait-based”) assessment is the pillar of the US policy of “de-regulation”, which explicitly exempts products from regulations. It focuses only on the intended outcome of a theoretical intervention into the genome, and ignores or denies the uncertainties and risks inherent in the genetic modification process and its real behaviour after release, as well as indirect negative impacts.

Proponents want to move from precaution to “proof of harm”

Accepting this drive for de-regulation would mean abolishing the EU regulatory approach, which is based on the Precautionary Principle. It would mean adopting or harmonizing with the US approach, which is based on deregulation and what we term here the “proof-of-harm” principle. This means putting the burden of proof of harm on the shoulders of those who are harmed. In this view, harm and its causal link to the product or process in question must effectively be scientifically proven to a high standard by the victims. Yet we suggest that, consistent with the European Environment Agency’s thoroughly evidence-based approach⁶, the developers, promoters or beneficiaries of the process should be required to demonstrate that rigorous independent scientific research across all relevant health and environmental sustainability dimensions has shown no evidence of harm.

Arguments put forward by NGMT proponents to justify their position

The following key points are used to argue for deregulation, exemption or “light-touch, product-based” regulation of organisms and products developed through NGMTs for environmental applications:

- Only the intended trait present in the end product of the NGMT “event(s)”⁷ should be considered by regulators, and no attention should be given to the

⁵ A threshold of up to 18 base pairs has been suggested.

⁶ European Environment Agency, *Late Lessons from Early Warnings: The Precautionary Principle in the 20th Century*, 2001, Copenhagen; vol.2, *Science, Precaution, Innovation*, 2013, Copenhagen, <https://www.eea.europa.eu/publications/late-lessons-2/download>

⁷ A genetic modification is often referred to as an “event” happening in the DNA or RNA. By extension, the word “event” is also used for the GMO resulting from one single GM effort; if

processes by which these “events” were created within the entire organism, whether a virus, microbe, plant or animal.

- In the majority of NGMT events, foreign DNA is not present at the end of the manipulation.
- The small DNA base unit changes brought about by genome editing methods, which either knock-out (ablate, deactivate) a gene or modify the function of a gene’s protein or RNA product, can mimic what may occur naturally through random mutation, i.e. without human intervention.
- The intended changes in the DNA or RNA are precise and singular, i.e. few or no other genome alterations occur in target organisms.
- The outcome of the NGMT “event(s)” is predictable and the intended changes will not interact with other genes or pathways or the organism as a whole. Therefore the products derived from these processes are safe, whether they are food products or organisms belonging to an agricultural or environmental system.

The undersigned do not accept these claims

We, the undersigned, challenge these claims as scientifically unjustified. We contend that NGMTs are indeed genetic modification techniques (as they do modify genetic material or gene function regulation via epigenetic or other changes) and that organisms produced by these methods are therefore, logically, genetically modified organisms (GMOs).

We assert that the application of these techniques allows for outcomes that may be unprecedented in human experience:

- Even accepting that some products of these techniques might be indistinguishable from organisms that have arisen without human intervention, they are not necessarily so, nor does this history matter for protecting the public.
- These techniques may be applied in a series of incremental changes, any number of which could be indistinguishable from those arising individually in nature, but collectively be entirely unknown to Earth. Genome editing NGMTs are being developed to be used simultaneously and/or sequentially. This allows either the simultaneous modification of multiple genetic sequences or the sequential modification of a single or different genetic sequence(s)⁸. Hence, even in cases where each change made is individually small, the totality of changes applied could produce an organism that is substantially different from the non-GM original. Such an organism may be as different

the same effort is repeated, the result is a different event (with another name), since GM is not predictable.

⁸ Khurshid H, Jan SA, Shinwari ZK, Jamal M, Shah SH (2017). An era of CRISPR/ Cas9 mediated plant genome editing. *Curr Issues Mol Biol.* 26: 47-54.

from a parental line as any organism produced with “conventional” transgenic genetic modification techniques, or even more so.

- The general claim that genomes changed using an NGMT are always identical to those that would arise without human intervention at the molecular level is unproven and undocumented scientifically. Using only an examination of one outcome of the series of interventions, the intended nucleotide sequence, is not valid as a final proof of the claim.
- Even if no foreign DNA remains in the end product, the intended genetic or epigenetic change in the organism's own DNA or RNA is detectable.
- Off-target, unintended changes in the genome occur frequently when these techniques are applied to some organisms and have not been excluded as happening in any organism, to our knowledge. This has been documented in published research, especially in the case of the genome editing NGMTs^{9,10}. Unexpected patterns of mutations induced by genome editing NGMTs at both on-target and off-target sites have recently been described^{11,12,13}. These findings indicate that we do not yet know all the mechanisms by which these methods bring about changes in the sequence of DNA, nor to what extent these may differ between animals and plants, or subgroups. This undermines our ability to fully predict the outcomes of these procedures. Whilst different papers may use different terms¹⁴, the currently recognised off-target effects include:
 - Unintended effects resulting from the intended alteration. For example, if the alteration has changed the activity or specificity of an enzyme, this can result in its carrying out or giving rise to biochemical reactions other than those intended.
 - Unintended alterations or mutations to other DNA or RNA sequences in addition to the target sequence(s). These off-target effects have often been documented^{15,16,17,18}. In cases where they have not been found,

⁹ Yee JK (2016). Off-target effects of engineered nucleases. FEBS J. 283: 3239-3248. doi: 10.1111/febs.13760

¹⁰ Bortesi L et al. (2016). Patterns of CRISPR/Cas activity in plants, animals and microbes. Plant Biotechnol J. 14: 2203-2216. doi: 10.1111/pbi.12634

¹¹ Schaefer KA et al. (2017). Unexpected mutations after CRISPR-Cas9 editing *in vivo*. Nat Methods 14: 547-548. doi: 10.1038/nmeth.4293

¹² Shin HY et al. (2017). CRISPR/Cas9 targeting events cause complex deletions and insertions at 17 sites in the mouse genome. Nature Commun. 8: 15464. doi: 10.1038/ncomms15464

¹³ Mou H et al. (2017). CRISPR/Cas9-mediated genome editing induces exon skipping by alternative splicing or exon deletion. Genome Biol. 18:108. doi: 10.1186/s13059-017-1237-8

¹⁴ E.g. unintended, unanticipated, off-target, non-target or unpredicted effects.

Depending on the authors, these terms may differ in meaning or overlap. They also get pooled into “off-target” effects by some, which is the meaning we use here.

¹⁵ Yee JK (2016). Off-target effects of engineered nucleases. FEBS Journal 283: 3239-3248

¹⁶ Bortesi L et al. (2016). Patterns of CRISPR/Cas activity in plants, animals and microbes. Plant Biotechnol J. 14: 2203-2216

the genomic DNA has usually not been sequenced as a whole to check for them¹⁹.

Off-target effects at a DNA, RNA or protein level can lead to unintended alterations in the biochemistry of the organism. This is the case even when no foreign DNA is present at the end of the NGMT manipulation. In the case of plant foods produced with these techniques, off-target effects can lead to unexpected toxins or allergens, or altered or compromised nutritional value. Even non-GMO plants are efficient at producing their own toxins – for example, to defend themselves against pests. The radical nature of the changes that can be introduced by NGMTs could result in unexpectedly high levels of such toxins or in the production of novel toxins. Ecological concerns have been raised regarding unintended effects of environmental release of NGMT products in the target and non-target wild organisms, crops and livestock, the difficulties in predicting those effects in the complexity of the natural ecological context, and corresponding uncertainties in risk assessment and risk management^{20,21,22} and related ethical issues²³.

- The concept of gene drives is a special case of NGMT (here CRISPR) application, because it intentionally reverses the idea of preventing the spread of genetic modifications to wider populations or non-target organisms. On the contrary, gene drives are designed to promote the spread of genetic modifications to complete populations in the wild and can even do so to entire species on a global scale. This includes the intentional extinction of populations or entire species, currently suggested for mice, insects (mosquitoes, flies), agricultural pests and invasive species. Rather than addressing root causes, e.g. poor sanitation or inappropriate agricultural or conservation practices, such approaches may exacerbate problems, or give

¹⁷ Shin HY et al. (2017). CRISPR/Cas9 targeting events cause complex deletions and insertions at 17 sites in the mouse genome. *Nature Communications* 8, Article number: 15464.

¹⁸ Mou H et al. (2017). CRISPR/Cas9-mediated genome editing induces exon skipping by alternative splicing or exon deletion. *Genome Biology* 18:108.

¹⁹ It has been shown that NGMT procedures may lead to unexpected and unintended mutations, and that such mutations do not only occur in specific sequences, predicted through specific computer algorithms, but also at unpredicted locations. In addition, longer ‘guide sequences’ (a tool used in some techniques) that would be expected to improve the precision of the process, do not reduce or may even exacerbate these off-target effects.

²⁰ Oye KA, Esvelt K, Appleton E, Catteruccia F, Church G, Kuiken T, Lightfoot SB-Y, McNamara J, Smidler A, Collins JP (2014) Regulating gene drives. *Science* 345(6197): 626-628. doi: 10.1126/science.1254287

²¹ Rodriguez E (2016) Ethical issues in genome editing using Crispr/Cas9 system. *J Clin Res Bioeth* 7:266. doi:10.4172/2155-9627.1000266

²² Nuffield Council on Bioethics (2016) Genome editing. An ethical review. London. <https://nuffieldbioethics.org/wp-content/uploads/Genome-editing-an-ethical-review.pdf>

²³ Jasanoff S (2015) CRISPR democracy: Gene editing and the need for inclusive deliberation. *Issues Sci Technol* 32(1):25-32. <http://issues.org/32-1/crispr-democracy-gene-editing-and-the-need-for-inclusive-deliberation>

rise to new and different ones. At best symptoms are treated while the causes are left intact. In addition, the risk of causing ecological imbalance and disruption is high. To eradicate insect species, for example, will have complex indirect effects on whole ecosystems, altering or disrupting food chains and associated biodiversity and potentially ecosystem function (e.g. pollinators may be harmed). Furthermore, there is a growing body of evidence suggesting that these approaches are not sustainable solutions: e.g. resistance rapidly evolves in insects targeted by gene drives in an effort to eradicate or reduce them (e.g. pathogen-carrying mosquitoes)^{24,25,26}. Finally, the boundaries delimiting the flow of genetic materials within populations and species are well known to be only partial, making it highly likely that population-scale gene-drives in a target population will escape to non-target populations. At the species level, gene drives intended to destroy an undesirable species are also likely to move, once released into the environment, into desirable relatives of the target species. For example, a gene drive to destroy a weed species would very likely cross over to related crop species, with potentially devastating consequences for humans.

The reality of NGMTs requires precaution

The above facts are clear indications of potential serious and irreversible harm. In spite of the scientific uncertainty involved, action must urgently be taken to prevent such harm. This is precisely what constitutes the Precautionary Principle. The Precautionary Principle is a fundamental element not only of EU legislation but also of the Convention on Biological Diversity (CBD) and its Cartagena Protocol on Biosafety. The Protocol puts the Precautionary Principle into operation through its substantive provisions.

It is important to recall that the Precautionary Principle was not born out of risk aversion, but out of a history of “late lessons from early warnings.”²⁷ When looking at precaution in the context of GMOs, we have to remember that these

²⁴ Zentner GE and Wade MJ (2017). The promise and peril of CRISPR gene drives: Genetic variation and inbreeding may impede the propagation of gene drives based on the CRISPR genome editing technology. *Bioessays*. 1 September. doi: 10.1002/bies.201700109.

²⁵ Unckless RL, Clark AG, Messer PW (2017). Evolution of resistance against CRISPR/Cas9 gene drive. *Genetics* 205: 827-841. doi: 10.1534/genetics.116.197285

²⁶ Callaway, E (2017). Gene drives thwarted by emergence of resistant organisms. *Nature* 542(7639). doi: 10.1038/542015a. <http://www.nature.com/news/gene-drives-thwarted-by-emergence-of-resistant-organisms-1.21397>

²⁷ There is a report of the same name, documenting (in two volumes) many cases where early indications of harm from various technologies were neglected with serious consequences: European Environment Agency, *Late Lessons from Early Warnings: The Precautionary Principle 1896-2000*, 2001, Copenhagen, http://www.eea.europa.eu/publications/environmental_issue_report_2001_22; vol.2, *Science, Precaution, Innovation*, 2013, Copenhagen, <https://www.eea.europa.eu/publications/late-lessons-2/download>

organisms are living systems with the ability to self-replicate and spread their modified genes, far and wide.²⁸ As has only recently been brought into understanding, even relatively precise genomic interventions can result in uncontrolled and unpredictable, thus unforeseen behavioural effects, since the systemic complexity of the organismic system being manipulated generates variable effects depending on precise conditions which are not at all scientifically fully understood. Thus additional levels of uncertainties and risk are created by such new techniques, in contradiction of the claim to greater precision and control.

All products of NGMTs must therefore be regulated at the level of strictest GMO regulations, and new, technique-specific regulations may be necessary

The scientific facts outlined above convince us that all products of NGMTs should be regulated at least as stringently as is currently required by the strictest GMO regulations (for example, the European Union regulations) and as permitted by the Cartagena Protocol on Biosafety and Codex Alimentarius.

While there is much scope to improve even the strictest existing GMO regulations, this is outside the remit of this present statement.

Some NGMTs can be used to radically alter an organism, completely changing or eliminating specific metabolic pathways. Such products would require highly stringent regulation. Pronounced changes could for example be made by multiple applications of small base unit genome editing, either in parallel or in sequence. A series of such small base unit changes in different genetic sequences can be designed to modify whole metabolic pathways.

NGMT products may also in some cases closely resemble “conventional” transgenic GM products. In these cases, if NGMT-derived organisms were exempted from the regulations applied to transgenic GMOs, then the former would escape regulation, but the latter would be regulated. This regulatory anomaly could threaten public trust in food safety, environmental safety and regulation.

All GMOs and their products, whether derived from “conventional” GM or NGMTs, from seed to table, should be labelled in order to ensure consumer and farmer choice and to enable traceability, monitoring and regulatory oversight in the case of any adverse effects that appear post-commercialization. Traceability

28 Steinbrecher R and Paul H (2017). New Genetic Engineering Techniques: Precaution, Risk, and the Need to Develop Prior Societal Technology Assessment. *Environment: Science and Policy for Sustainable Development*. 59(5):38-47

and labelling are also minimum requirements for being able to assign causation and responsibility in the event of long-term adverse effects.

To the extent that NGMTs provide improved capacities to rapidly produce large numbers of GMO products, new standards and thresholds may be necessary to regulate their amplified potential consequences (in addition to e.g. existing EU regulations). Some methods of NGMT (e.g. CRISPR-Cas9) make it possible to massively miniaturize and automate the production of GMO life-forms, especially in microbes. This augmentation in quantity, when translated into the possibility of massively increased releases of GMOs, may well represent a new threshold requiring qualitatively improved, stricter regulatory standards.

DNA sequencing should not be confined to predicted off-target sites

It is not sufficient to regulate organisms created by the genome editing class of NGMTs on the basis of DNA sequencing that looks only at anticipated off-target sites that are predicted by e.g. computer programs solely on the basis of similarity of their base unit sequences to the intended target site. Off-target sites are not limited to such sites of similarity. Genome editing tools have been shown to generate DNA cuts at unexpected locations that are substantially dissimilar to the intended target site, resulting in base unit substitutions, insertions and deletions²⁹.

Furthermore, direct transformation processes³⁰ and plant tissue culture³¹ both give rise to large numbers of random mutations in the resulting genetically modified plant³². This is also true for transformation of plants with CRISPR/Cas, which was found to result in unintentional and random integration of bacterial vector backbone DNA into the plant genome^{33,34}. The increased use of protoplasts is adding to such process-induced mutations. All these also need to be considered. Yet the claim that the new techniques are more precise therefore more controlled, and that this justifies no regulation of the process, only of the final product, neglects all the scientific evidence summarized above.

²⁹ Fu Y et al. (2013). High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells. *Nat Biotechnol.* 31: 822-826. doi: 10.1038/nbt.2623

³⁰ Transformation is the insertion of DNA into a living cell.

³¹ Plant tissue culture is the method by which plant tissues (and eventually whole plants) are raised from single (genetically modified) cells. It is thus an obligatory part of the genome editing procedure with plants.

³² Wilson AK et al. (2006). Transformation-induced mutations in transgenic plants. *Biotechnol. and Genetic Eng. Rev.*, 23: 209-237 doi: 10.1080/02648725.2006.10648085

³³ Braatz, J., et al. (2017). "CRISPR-Cas9 Targeted Mutagenesis Leads to Simultaneous Modification of Different Homoeologous Gene Copies in Polyploid Oilseed Rape (*Brassica napus*)." *Plant Physiol.* 174(2): 935-942. doi: 10.1104/pp.17.00426

³⁴ Li WX, Wu SL, Liu YH, Jin GL, Zhao HJ, Fan LJ, Shu QY (2016) Genome-wide profiling of genetic variation in *Agrobacterium*-transformed rice plants. *J Zhejiang Univ Sci B* 17: 992–996 doi: 10.1631/jzus.B1600301

Biohacking, bioterror and dual use

Genome editing NGMTs are much easier and cheaper to use than “conventional” transgenic genetic modification techniques. “Garage scientists” or biohackers can now obtain genome editing kits on the internet and produce their own genome-edited products. This is already happening^{35,36} and constitutes a serious consequence of these techniques. Just one genetic modification can transform a harmless bacterium into a pathogenic or antibiotic-resistant bacterium. This and other applications of genome editing techniques have become so easy to realise, that they open up the possibility of abuse and inadvertent misuse with an alarming likelihood.

Academic and government scientists have pointed out^{37,38} that if genome editing techniques are not strictly regulated, the potential for inadvertent harm as well as for acts of bioterror will increase exponentially.

Process-based and product-based regulation must be applied

Given that NGMTs:

- Use laboratory-based, artificial DNA and RNA modification procedures³⁹
- Do not in themselves involve natural cross-breeding
- Result in intended alterations in the function or activity of one or more DNA or RNA sequences that become inherited⁴⁰,
- Cause unintended and/or unpredictable off-target effects, and
- Are in some cases easy and cheap to use,

the regulations applied to their products should be process-based as well as product-based, as with the current EU GMO regulations. The claim that, because of their greater precision, the new GM techniques create only intended and predicted effects on the new plant-products they generate, and no unpredicted effects, is spurious.

³⁵ Regalado, A (2016). Top U.S. Intelligence Official Calls Gene Editing a WMD Threat. MIT Technol Rev, 29 February. <https://www.technologyreview.com/s/600774/top-us-intelligence-official-calls-gene-editing-a-wmd-threat/>

³⁶ Marcus, Amy Dockser (2017). DIY gene editing: Fast, cheap—and worrisome. The Wall Street Journal. 26 February. <https://www.wsj.com/articles/diy-gene-editing-fast-cheap-and-worrisome-1488164820>

³⁷ Mullin, Emily (2016). Obama advisers urge action against CRISPR bioterror threat. MIT Technology Review. 17 November. <https://www.technologyreview.com/s/602934/obama-advisers-urge-action-against-crispr-bioterror-threat/>

³⁸ Yuhas, Alan and Kelkar, Kamala (2016). 'Rogue scientists' could exploit gene editing technology, experts warn. The Guardian. 12 February. <https://www.theguardian.com/science/2016/feb/12/rogue-scientists-could-exploit-gene-editing-technology-experts-warn>

³⁹ This characteristic meets the definition of “modern biotechnology” used by Codex Alimentarius, since these procedures involve “application of *in-vitro* nucleic acid techniques.”

⁴⁰ Exception: in transgrafting, the genetic alteration may not be inherited.

Unlike product-based-only regulation, process-based regulation is capable of highlighting the mechanisms by which unintended and off-target gene function disruption effects can take place. Thus, process-based regulation is true to the state of this science and technology. Attempts to argue that such regulation is superfluous or excessive are therefore disingenuous and place an unacceptable risk onto public health, the environment and trade. By not requiring testing consistent with Codex Alimentarius, this could put EU products at risk in international markets, since countries that require full safety assessments for such techniques could reject exports from countries that do not require such safety assessments.

Conclusion

In conclusion, from a strictly scientific and technical perspective, NGMTs are clearly genetic modification procedures that result in the production of GMOs. Such techniques give rise to predictable as well as inadvertently generated risks when used in a context of agriculture, conservation or ecological management. Therefore the products of NGMTs in these contexts (viruses, microbes, plants and animals) should be at least as stringently regulated as the organisms produced with the transgenic methods used in currently commercialized GMOs. This would bring the regulation of NGMT applications in agricultural and other contexts into line with their recognition in the sphere of medical research, where they are unquestionably considered as genetic modification. It would also be in accordance with the EU Precautionary Principle. Contrary to the repeated claims of commercial interests threatened by it, the Precautionary Principle does not require an impossible proof of safety prior to regulatory acceptance, but instead requires scientifically independent, searching and sustained examination of the questions of harm from such products, with the injunction to intervene even where scientific proof of harm is incomplete, if there are reasonable scientific grounds to suppose potential harm from the processes involved. First of all, this requires that the processes involved are themselves subject to regulatory appraisal and not only their products. Secondly, when the evidence shows, as cited above, that these processes do not control unintended and unpredicted – and potentially harmful – consequences, as their proponents claim they do, then the case for their thorough and scientifically independent risk appraisal is beyond argument.

The document was first signed by 60 scientists and experts.

The document is now open for further signatures; scientists with a PhD and physicians (with or without PhD) agreeing with the content are invited to sign the statement at: www.ensser.org.

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