

Recombinant DNA: 50 Years of Innovation, Impact and Controversy

Markus Wyss, DSM Nutritional Products Ltd., Kaiseraugst, Switzerland

Hanna Abbas Lindfors, DSM Food Specialties B.V., Delft, the Netherlands

Alexandra Lensch, Evonik Operations GmbH, Nutrition & Care, Hanau, Germany

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Abstract

Recombinant DNA technology has had profound impacts on life science research, agriculture, diagnostics and cures, and the development of a myriad of commercial products. This article sheds light on the historical context and on the flabbergasting technological developments made over the past five decades. In order to leverage the full potential of recombinant DNA technology – and of genetic engineering more generally – in addressing the current environmental and socio-economic challenges, it will be crucial to bring the regulatory framework conditions in sync with the scientific state-of-the-art.

Key Points

- Recombinant DNA technology has had a transformative impact on science and business.
- Genetic engineering is expected to be an important tool in addressing food insecurity, climate change and the need to transition to a more sustainable, circular economy.
- Most of the initial safety concerns raised in the 1970s have dissipated into thin air, but still influence public perception today.
- To leverage the full potential of recombinant DNA technology and genetic engineering, the public concerns need to be addressed, and the regulatory framework conditions need to be updated to the scientific state-of-the-art.

Introduction

Recombinant DNA has revolutionized many areas of research and has opened up a myriad of industrial applications. In the ensuing sections, we will (i) provide a definition of the term; (ii) place recombinant DNA in the historical context of genetic modification; (iii) highlight illustrative examples of how recombinant DNA benefits people, society and the environment; (iv) assess the safety and regulatory implications of recombinant DNA; and (v) conclude with some key recommendations.

An Attempt to Define Recombinant DNA

For “recombinant DNA”, there is no globally accepted definition. The Cartagena Protocol on Biosafety ([Secretariat of the Convention on Biological Diversity, 2000](#)) defines a “living modified organism” (LMO) as any living organism that possesses a novel combination of genetic material obtained through the use of “modern biotechnology”. It continues to specify that “modern biotechnology” means the application of, among others, “in vitro nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA)”, but fails to provide a meaningful definition of the latter term.

We propose the following definition, which will guide us through the remainder of this article: Recombinant DNA is the result of an intended recombination of two or more pre-defined stretches of DNA, either from the same organism or from different organisms, to afford new or improved properties that are of value to science, medicine, agriculture, and/or industry. The intended recombination event(s) can be introduced in a targeted (e.g., DNA cloning by homologous recombination, or SDN-2 or SDN-3 genome editing techniques) or non-targeted manner (e.g., random insertion of heterologous sequences).

According to this definition, classical mutagenesis – i.e. the use of chemical mutagens or UV irradiation to induce random mutations, followed by the selection of improved mutants – will not result in recombinant DNA, because the DNA modifications are random even if selected, rather than the result of an intended recombination. The same applies to mutations introduced by SDN-1 genome editing techniques. Although with SDN-1, the genetic locus to be modified is predefined, the nature of the change itself is still random.

Recombinant DNA techniques can be used to introduce, e.g., a single base substitution or an entire heterologous gene into a target genome. In the latter case, the recombination of DNA is entirely evident in the organism's genome. However, a single base substitution could be the result of natural evolution, classical mutagenesis, genome editing, or recombinant DNA technology. To conclude, not every genetically modified organism contains recombinant DNA. The other way round: even if recombinant DNA is used in the process of genetic modification, the final product – i.e. the modified organism – may no longer be identifiable as a product of recombinant DNA technology.

Some Historical Context

Ever since humans became sedentary some 10,000–20,000 years ago, they improved their food sources – either intentionally or non-intentionally. Initially, this was done by the selection of desired, favorable traits, such as large fruit size, high grain number, and good taste. The discovery of Mendelian inheritance opened the door to the breeding of improved plant varieties or animal breeds. Still, breeding is a tedious and slow process, particularly for species with long generation times. Around 1930, classical mutagenesis was introduced, i.e. the use of gamma or X-ray irradiation or of chemical mutagens to deliberately induce random mutations in a target organism and thereby to “accelerate” evolution. It is in the very nature of random classical mutagenesis that next to a few mutations leading to improvements in a desired trait, a large surplus of neutral or even deleterious mutations are generated. Thus, the few beneficial mutations need to be identified in extensive screening campaigns, and the neutral and deleterious mutations often need to be eliminated by back-crossing of the desired trait into “clean”, non-mutagenized varieties or breeds.

But science further advanced. As eloquently outlined by [Berg and Mertz \(2010\)](#), the key methodological advances that allowed the emergence of recombinant DNA technology in the 1970s were: “(i) the discovery of enzymes that modify DNA molecules in ways that enable them to be joined together in new combinations; (ii) the demonstration that DNA molecules can be cloned, propagated, and expressed in bacteria; (iii) the development of methods for chemically synthesizing and sequencing DNA molecules; and (iv) the development of the polymerase chain reaction (PCR) method for amplifying DNA in vitro”. This allowed sequences from different organisms to be pasted together in desired combinations, to incorporate them into bacteriophages (“vectors”) or extrachromosomal plasmids, and to use those vectors or plasmids to introduce the designed DNA construct into a target organism. Since the efficiency of the initial methods was still low, a desired trait (i.e. the designed DNA construct) was often combined with an antibiotic resistance gene, so that the antibiotic resistance trait could be used for the straightforward selection of those few organisms which had acquired the desired DNA construct.

The efficiency of the methods improved over time, and the use of antibiotic resistance markers is either no longer required, or they can be easily removed in organisms to be used commercially. Most relevant, however, was the emergence – as of 1985 – of the so-called genome editing techniques (also called new breeding techniques) such as CRISPR-Cas ([Tröder and Zevnik, 2022](#); [Matsumoto and Nomura, 2023](#)). These techniques afforded a further boost in precision and efficiency of genetic engineering, translating into lower R&D costs, and leading to the “democratization” of biotechnology, i.e. making it amenable to a broader range of actors, including the Do-It-Yourself community ([Kuiken, 2016](#); [Jackson *et al.*, 2019](#)). Genome editing techniques, in the most subtle way, allow the introduction of a single base substitution in an entire genome, which would make the change indistinguishable from what could happen through natural evolution or through the use of classical mutagenesis. However, these genome editing techniques can also be used to afford more complex changes, such as the re-design and/or introduction of heterologous sequences. Therefore, these techniques seriously question the concept of “genetically modified organisms” and of “recombinant DNA”, both from a scientific and from a regulatory perspective (see [Fig. 1](#) and below).

Benefits and Promises of Recombinant DNA Technology

[Berg and Mertz \(2010\)](#) conclude their article mentioned earlier by emphasizing (i) that the most significant contribution of recombinant DNA technology has been the “great strides made in understanding fundamental life processes and the ability to investigate problems that had previously been unapproachable” and (ii) the appreciation from myriad investigations that “nothing in the man-made world rivals the complexity and diversity of this earth’s organisms”. Indeed, the targeted overexpression or deletion of genes has been and still is fundamental in deciphering the functions of the proteins they encode – for instance by using transgenic knock-out or knock-in mice to understand human disease (e.g., [Doyle *et al.*, 2012](#); [Kersten *et al.*, 2017](#)).

Commercially, one of the best-known examples are genetically engineered (“transgenic”) crops (e.g., [ISAAA, 2020](#); [Brookes, 2022](#)). Genetically engineered crops are cultivated in 29 countries, on 186 million hectares of land, which is equivalent to the size of Sudan and represents close to 4% of the global agricultural area. Another approximately 40 countries are not cultivating genetically engineered crops themselves, but are importing such crops, primarily for livestock feeding purposes ([van Eenennaam, 2013](#)). Just four

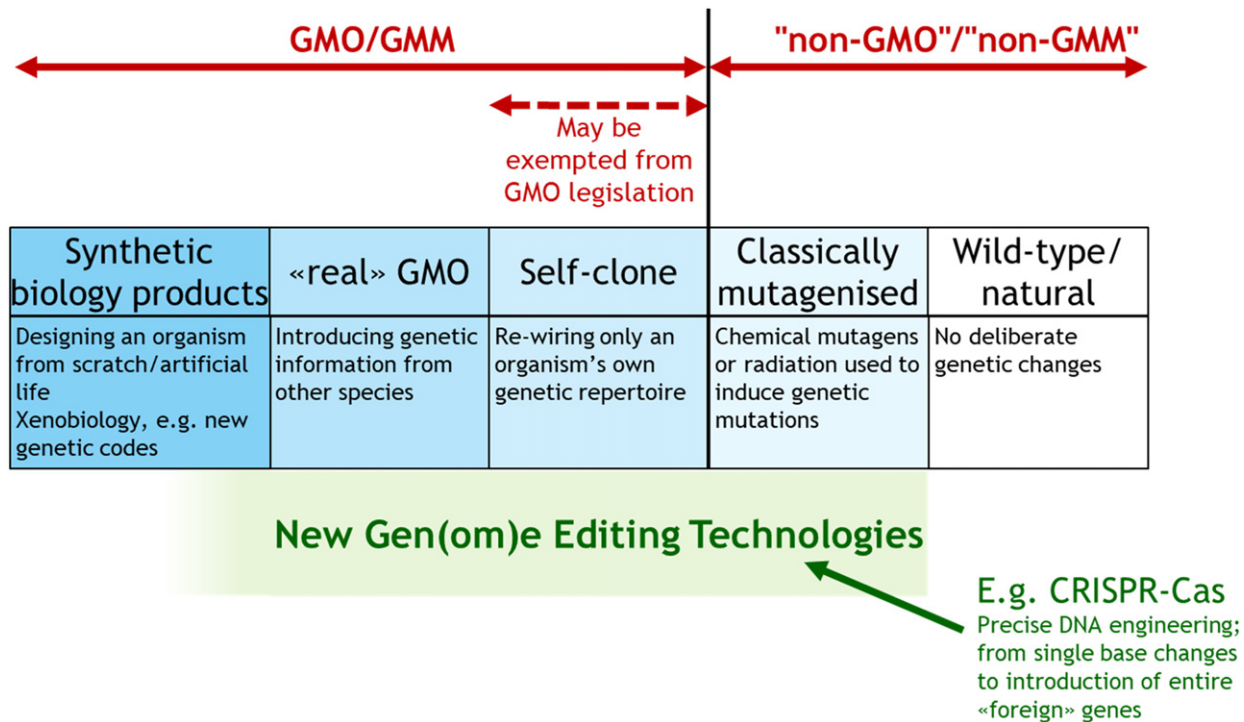


Fig. 1 Graphical representation highlighting that a distinction between “genetically modified” and “natural” organisms is arbitrary and misleading, and that there are many more shades of gray regarding the nature of genetic changes which can occur naturally and/or be engineered intentionally. In addition, the scheme demonstrates that regulatory authorization systems that are based on the technologies applied bear the risk that similar products obtained by different technologies are subject to very different regulatory burden.

crops make up roughly 99% of all genetically engineered plants grown globally: soybeans, maize, cotton, and canola; just over 47% of the global area of these four crops were planted with genetically engineered varieties in 2020. The main traits introduced into these plants are herbicide resistance and insect resistance, whereas other traits, including changes in the nutritional profile of food crops, still provide only marginal contributions. The main reasons for the focus on just four crops and on two main traits are the high regulatory burden and the associated high costs (115 mio. USD) and long timespans (16.5 years) to bring a genetically engineered crop to market (AgbioInvestor, 2022). These high burdens can typically be afforded only by large, multinational seed companies. However, things are changing: (i) next to the four main crops, genetically engineered varieties are now also available for alfalfa, sugar beet, sugarcane, papaya, safflower, potato, eggplant, squash, apple, and pineapple; (ii) next to multinational seed companies, there are public sector initiatives to develop genetically engineered varieties of rice, banana, potato, wheat, chickpea, pigeon pea, and mustard with various quality traits that are beneficial to food producers and consumers in developing countries; (iii) the new genome editing (“new breeding”) techniques are more efficient, more precise, and less costly in application, thereby making engineering of improved crops more affordable for a broader range of actors; and (iv) some countries have already taken decisions to reduce regulatory burden for at least some crops obtained with genome editing techniques (Sprink et al., 2022).

Another important field of use of recombinant DNA technology is the production of biopharmaceuticals (Nambisan, 2017). They can be produced by genetically engineered plants on agricultural lands (Eidenberger et al., 2023), by genetically engineered microbes in industrial fermentation processes (e.g., Riggs, 2021), or by genetically engineered cell lines of higher eukaryotes in tissue culture (mainly Chinese Hamster Ovary cells; (Szkodny and Lee, 2022)). An important class are therapeutic (monoclonal) antibodies, which alone were valued at 115 billion USD in 2018 and were predicted to reach 300 billion USD by 2025 (Lu et al., 2020). Other examples worth mentioning may be recombinant hepatitis B vaccines produced by genetically engineered baker's yeast (Keating and Noble, 2003) or injectable insulin for diabetics produced by genetically engineered *Escherichia coli* (Riggs, 2021). Recombinant DNA technology is also applied in human gene therapy, defined as the transfer of DNA to somatic cells of a patient to either correct a genetic defect or to overexpress proteins that are therapeutically useful (Danaeifar, 2023).

Genetically engineered microbes are also used in industrial fermentation processes for a broad range of other markets. For instance, they can be used for mass production of laundry or dishwashing enzymes that provide better cleaning performance at lower wash temperatures (Olsen and Falholt, 1998). Or they can serve as sources of enzymes used in the production of human food or animal feed, and of food ingredients or feed additives such as amino acids, vitamins, colorants, omega-3 fatty acids, non-caloric sweeteners, flavors or flavor enhancers, human milk oligosaccharides, and vegan alternatives of dairy and other animal-derived products (Lensch et al., 2022; Augustin et al., 2023). In addition, they can be used to ensure the safety of food, for instance through the production of enzymes that degrade or prevent the formation of contaminants such as mycotoxins or acrylamide, or

degrade food allergens such as gluten or lactose. Increasingly, industrial fermentation processes can also be used for the low-cost production of bulk chemicals such as 1,3-propanediol (Kurian, 2005) or farnesene (Hill *et al.*, 2020).

Looking towards the future, genetic engineering is destined to be even more crucial for addressing the socio-economic and environmental challenges that our planet is facing, and for the transition from fossil-based to renewable raw materials, and from a mostly linear to a circular economy in which raw material consumption and waste generation are minimized and the sustainability of the global economy is drastically improved. As examples, genetic engineering is expected to play a pivotal role in the production of second-generation biofuels (Lu *et al.*, 2022) or in bioremediation (Hou *et al.*, 2020; see also Khan *et al.*, 2016). Finally, modifying bacteria and yeast so that they can perform photosynthesis, i.e. use the energy of sunlight to assimilate CO₂ from the atmosphere and transform it into products of interest including biofuels, is a promising prospect for reducing or even reverting carbon emissions and for alleviating the energy and climate crises (Kim *et al.*, 2022; Meng *et al.*, 2022).

In conclusion, recombinant DNA technology and/or genetic engineering are not the solution to all the challenges and problems that our planet is facing; however, they have an outstanding potential to contribute significantly to a more sustainable future and to achieve our ambitions in addressing climate change, biodiversity loss or food insecurity – provided that regulatory framework conditions and public opinion support rather than impede innovation in this space.

Evolution of the Regulatory Landscape – It is Time for Revolution

With the emergence of recombinant DNA technology in the 1970s, concerns were raised that unrestricted use of this new and unknown technology could lead to unforeseen and damaging consequences for human health and the Earth's ecosystem (Berg and Singer, 1995). This led prominent scientists to organize two landmark meetings at the Asilomar Conference Center in Pacific Grove, California, one in January 1973 and the other in February 1975, to discuss the potential risks of the technology (Frederickson, 1991; Berg and Mertz, 2010). They issued several recommendations – including the prohibition of pipetting by mouth, and the use of laminar flow hoods during all manipulations involving potentially infectious material – and agreed also on a temporary moratorium on some experiments. The discussions did not stay in the scientific community, but spilled over to the public and into politics, with heated debates and injunctions in the courts to forbid all such experimentation. Right after the second Asilomar conference, the National Institutes of Health (NIH) Recombinant DNA Advisory Committee was formed which issued the first edition of the *NIH Guidelines for Research Involving Recombinant DNA Molecules* in June 1976 (Wivel, 2014).

Further key developments in defining the regulatory frameworks to govern research and product development involving recombinant DNA included the publication of the OECD Blue Book in 1986 (Schiemann, 2006), the entry into force of the Genetically Modified Organism (GMO) directives 90/219/EEC (on the contained use of genetically modified micro-organisms) and 90/220/EEC (on the deliberate release into the environment of genetically modified organisms) in the European Economic Community in 1990 (EEC, 1990a,b), and the adoption of the Cartagena Protocol on Biosafety in 2000 (Secretariat of the Convention on Biological Diversity, 2000). All these documents are deeply rooted in the precautionary mindset of the early days of recombinant DNA technology, where the main (hypothetical) safety concerns were associated with the process of genetic engineering. And this in turn has led to a binary distinction between GMOs (organisms developed through recombinant DNA techniques) and non-GMOs. In the European Directives, a GMO is defined as “an organism in which the genetic material has been altered in a way that *does not occur naturally by mating and/or natural recombination*”, whereas in the Cartagena Protocol, a Living Modified Organism (LMO) is defined as “any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology”, whereby modern biotechnology is defined as “the application of:

- (a) In vitro nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles, or
- (b) Fusion of cells beyond the taxonomic family,

that *overcome natural physiological reproductive or recombination barriers* and that are not techniques used in traditional breeding and selection”.

Unfortunately, a binary classification system – GMO vs. non-GMO (or LMO vs. non-LMO) – is an extremely fertile ground for stigmatization as good versus bad, for emotional rather than rational, fact-based debates, and for departure into categorical, irreconcilable positions. And this, in turn, has prevented for decades that meaningful updates to these regulations could be made.

However, many things have changed since the early days of recombinant DNA technology. (i) Most importantly, the initial safety concerns dissolved into thin air. In the “decades since adoption of these various regulations for conducting recombinant DNA research, many millions of experiments have been performed without reported incident. No documented hazard to public health has ever been attributable to the applications of recombinant DNA technology” (Berg and Mertz, 2010). (ii) With technological advances, it has become increasingly evident that a binary distinction between GMOs and non-GMOs is arbitrary, and that the reality is much more granular (see Fig. 1). In addition, a particular genetic change (for instance a single base change or a specific gene deletion) could either happen naturally, be induced by classical mutagenesis, be generated by recombinant DNA techniques, or by the use of genome editing techniques, resulting in the very same organism which, however, would be subject to different regulatory burden due to the technology applied. (iii) The analytical techniques have also improved dramatically. In particular, whole-genome sequencing is both feasible and affordable for prokaryotes and increasingly also for (higher) eukaryotes,

which allows the genetic changes – both those introduced intentionally and those potentially happening unintentionally – to be duly characterized, so that we can shift our focus from assessing the *process* towards characterizing the *product*. (iv) Crossing the species barrier has proven not to be a man-made violation of nature, but a normal natural phenomenon. Horizontal gene transfer, i.e. the transfer of DNA from one species to another, was demonstrated to naturally happen over often large evolutionary distances, for instance from plants to insects (Gilbert and Maumus, 2023) or from bacteria to animals (Husnik and McCutcheon, 2018). Thus, “recombination” is by no means an unambiguous decision criterion for the distinction between GMOs and non-GMOs. (v) Finally, all those who have attempted to use genetic engineering to tweak a microorganism to serve human interests will know how unruly they are, i.e. how many layers of control nature has built into the blueprint of life to secure survival and to prevent that things could get out of control.

We concur with Wivel (2014): “A conservative approach is entirely appropriate at the outset, but the acquisition of knowledge and experience forces a constant reevaluation of oversight paradigms”. For recombinant DNA technology and genetic engineering, we have gained so much knowledge and experience over the past five decades that it is high time to revisit the regulatory frameworks for biotechnological products. Very recently, first steps in this direction have been taken, by exempting – or attempting to exempt – at least some products of genome editing (or “new breeding techniques”) from the burdensome GMO approval process and the GMO labeling requirements that often deter customers and consumers of such products (Turnbull *et al.*, 2021; European Commission, 2023a; Jenkins *et al.*, 2023). However, this is not enough, since these exemptions are still rooted in the old, process-centric perspective that has proven to be crippling innovation, and therefore do not address the fundamental problem. What we truly and desperately need is to abandon the process-centric regulatory concepts distinguishing GMOs from non-GMOs, and to transition to a *product-centric* approval system where regulatory burden is proportionate to the potential risks of a given biotechnological product, focusing on the characteristics and the actual genetic modifications rather than on the methods how they have been achieved.

Recombinant DNA Should be of No Regulatory Relevance

Let us finish with a case in point where regulatory developments have derailed, resulting in a significant obstacle for innovation. Over the past 20 years, absence of recombinant DNA has increasingly been interpreted as a quasi-requirement for fermentation products to be used in human food and animal feed in the European Union. According to the current practice, absence of recombinant DNA needs to be confirmed in regulatory dossiers with an analytical test having a minimal sensitivity of 10 ng of genomic DNA per gram of product (EFSA, 2018), representing an impurity level of 0.000001%. Unfortunately, this development has spilled over to other geographies, e.g., Brazil (ANVISA, 2020) or India (2022), which now also request absence of recombinant DNA in fermentation products.

There are multiple reasons why, on a case-by-case basis, recombinant DNA *may* be considered in the safety assessment, but should *not* play a role in the regulatory classification of fermentation products:

- (i) Millions of experiments have demonstrated convincingly that there is no reason to believe that recombinant DNA is intrinsically unsafe.
- (ii) Accordingly, it is difficult to reconcile why for a generally safe impurity such as recombinant DNA, impurity levels below 0.000001% would be required, whereas for fumonisins, i.e. well-known mycotoxins, the maximum tolerated level in maize is 1 mg/kg = 0.0001%, i.e. 100-fold higher (European Commission, 2023b).
- (iii) The main hazard brought forward for recombinant DNA is the purported risk of horizontal gene transfer, which might “contaminate the environment” and, in the worst case, impact the safety of the product, particularly if a production strain used in fermentation contains acquired/introduced antibiotic resistance genes. However, a few clarifications are required here: first, producers of fermentation products have a strong preference and keen interest to work with intrinsically safe microorganisms such as baker’s yeast. Secondly, state-of-the-art production strains have the genetic modifications stably integrated into their chromosomes, and do not contain antibiotic resistance markers. Thirdly, residual recombinant DNA in fermentation products is present as naked DNA (i.e. not part of a living organism), which has a low probability of being taken up by other organisms. Fourthly, residual recombinant DNA in fermentation products used in food or feed is expected to be digested rapidly in the digestive tract. Fifth, horizontal gene transfer is a natural phenomenon. Thus, even in the unlikely case that uptake of residual recombinant DNA in fermentation products were to occur (e.g., by gut microbiota), it is most unlikely that novel combinations are created with hypothetical superpowers that nature would not have explored before. Sixth, all organisms defend themselves against neighbors and competitors, and one common instrument that bacteria have to do so is the production of antibiotics. Bacteria that produce antibiotics need to have mechanisms to protect themselves from their own antibiotics. The principal mechanisms of antibiotic resistance are modification and/or degradation of the antibiotic, its active removal (efflux) from the cells, its sequestration by special proteins, and the modification or bypass or protection of an antibiotic target (Peterson and Kaur, 2018). Thus, it is highly unlikely that all gut microbiota are free of antibiotic resistance genes, and that therefore residual recombinant DNA would represent a major contributor increasing the load of antibiotic resistance genes in the gut microflora. At the very least, detailed investigations would be warranted to corroborate whether residual DNA is a meaningful contributor altogether to the pool

of antibiotic resistance genes in human or animal gut microbiota. All in all, the purported risk of horizontal gene transfer from residual recombinant DNA in fermentation products is dramatically overrated.

- (iv) A requirement for absence of recombinant DNA for fermentation products would greatly hinder innovation as well as the transition to a green, circular economy. Imagine a feed additive such as an amino acid, vitamin or a feed enzyme produced by fermentation. Why should a producer purify the feed additive to a purity of – let's say – 95% and remove all proteins, lipids, carbohydrates, and other biomolecules, if such proteins, lipids, carbohydrates, etc., anyway need to be added to the feed of the animals? This would unnecessarily add costs, increase raw material use and waste generation, thereby compromise the sustainability of the product and - as a result - render it less attractive. A less purified product would, however, have higher levels of residual recombinant DNA, and it would seem ill-advised to impose incentives to remove or degrade safe recombinant DNA just to qualify as a contained-use fermentation product for which no GMO labeling is required.
- (v) Genetically engineered plants containing introduced antibiotic resistance genes have been and are still approved in a number of countries, and are considered safe (OECD, 2023). These plants contain loads of DNA, and it would be difficult to reconcile why residues of recombinant DNA in fermentation products should suddenly be considered less safe.
- (vi) Finally, in the European Union, there is currently no legal basis for a quasi-requirement for absence of recombinant DNA (Dederer, 2021; Lensch *et al.*, 2022).

In conclusion, the availability of very sensitive DNA analysis techniques should not be taken as a reason or excuse to impose obligations that are of no safety relevance. Instead, the learnings on the safety of recombinant DNA over the past five decades as well as the potential implications for innovation should be duly taken into account and result in decisions to drop absence of recombinant DNA as a generic regulatory requirement for fermentation products.

Conclusions

Recombinant DNA technology and genetic engineering more generally have had a phenomenal impact on life science research and already today offer a myriad of superior therapies as well as commercial products in a broad range of industries and applications, providing a wealth of benefits. In addition, these technologies are expected to make a major contribution in the transition towards a circular, bio-based economy, and in achieving the high ambitions related to sustainable food systems, climate change and biodiversity conservation – provided that the regulatory framework conditions support rather than impede innovation in this space.

Scientific developments have been exciting over the past decades, resulting in methods of genetic engineering that are much more efficient and precise, and that also allow much more detailed characterization of the products obtained, including the confirmation of their safety. On the regulatory side, scientific progress should *not* result in the strengthening of risk assessment, understood as making the requirements more and more demanding. Instead, scientific progress should indeed result in the strengthening of risk assessment, but understood as keeping requirements proportionate to real, *established* risks, focusing on the *real* safety concerns, and striking an appropriate balance between incentivizing innovation while securing the safety of the products. Accordingly, some current, questionable requirements like the one for absence of recombinant DNA should be let go.

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