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Review Article

Global trends in regulatory frameworks for animal genome editing in agriculture

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ABSTRACT Revolutionary advancements, such as the reduction in DNA sequencing costs and genome editing, have transformed biotechnology, fostering progress in manipulating biomolecules, engineering cells, and computational biology. Agriculture and food production have significantly benefited from tools like high-throughput microarrays, accelerating the selection of desired traits. Genetic engineering, especially utilizing genome editing, facilitates precise alterations in plants and animals, harnessing microbiomes and fostering lab-grown meat production to alleviate environmental pressures. The emergence of new biotechnologies, notably genome editing, underscores the necessity for regulatory frameworks governing LM (living modified) organisms. Global regulations overseeing genetically engineered or genome-edited (GE) organisms, encompassing animals, exhibit considerable diversity. Nonetheless, prevailing international regulatory trends typically exclude genome-edited plants and animals, employing novel biotechnological techniques, from GMO/LMO classification if they lack foreign genes and originate through natural mutations or traditional breeding programs. This comprehensive review scrutinizes ongoing risk and safety assessment cases, such as genome-edited beef cattle and fish in the USA and Japan. Furthermore, it investigates the limitations of existing regulations related to genome editing in Korea and evaluates newly proposed legislation, offering insights into the future trajectory of regulatory frameworks.

Keywords: agriculture, animal, genome editing, LMO

INTRODUCTION

The convergence of advancements in biological sciences alongside rapid progress in computing, automation, and artificial intelligence is propelling a new wave of innovation with far-reaching impacts across various sectors such as health, agriculture, consumer goods, and energy. While these advancements, which encompass gene editing and biological engineering, hold immense potential, they also entail significant risks. Particularly, our improving capacity to comprehend and manipulate biology has led to recent breakthroughs, notably the substantial reduction in DNA sequencing costs and the introduction of new methods like CRISPR for gene editing and cell reprogramming. Noteworthy advancements have been made in four specific areas: understanding and altering biomolecules, engineering cells, tissues, and organs within biosystems, bridging biology and machines through biomachines, and utilizing cells or molecules like DNA for computational purposes. Each area is progressing differently, transition-

ing from experimental stages to practical applications (Siebert et al., 2022).

Furthermore, advancements in agriculture, aquaculture, and food production have integrated technologies such as cost-effective, high-throughput microarrays, significantly expanding genetic data for plants and animals. This facilitates quicker and more cost-effective selection of desirable traits through marker-assisted breeding, outpacing the pace of traditional methods. Initially, genetic engineering in the 1990s introduced genetically modified organisms (GMOs) to enhance plant traits. Recent strides in genetic engineering, especially with tools like CRISPR, allow precise alterations within plants by using genes from compatible species or modifying gene combinations and regulatory sequences. These innovations extend to leveraging the microbiome of plants, soil, animals, and water to enhance agricultural productivity. Additionally, there is a growing focus on developing alternative proteins such as lab-grown meat to alleviate environmental pressures from standard livestock and seafood production. The cumulative impact of these advancements in agriculture and food production is estimated to range from $800 billion to $1.2 trillion over the next two decades, accounting for approximately 36 percent of the overall potential impact (Chui et al., 2023; Han et al., 2023; Lee et al., 2023)

NEW BIOTECHNOLOGY: GENOME EDITING

Various terms are employed to define regulations concerning LMOs or GMO across different countries. Among these, a prominent definition - outlined by the Secretariat of the Convention on Biological Diversity (SCBD, 2000) - describes a 'Living Modified Organism' as any living organism that possesses a novel combination of genetic material obtained by modern biotechnology. This includes *in vitro* nucleic acid techniques, such as recombinant deoxyribonucleic acid (rDNA) methods, direct injection of nucleic acid into cells or organelles, or cell fusion extending beyond the taxonomic family. These techniques surpass natural physiological reproductive or recombination barriers and stand apart from those utilized in traditional breeding and selection.

Gene editing involves molecular methods that precisely alter living organisms' genomes using site-directed nucleases (SDNs) to cut DNA at specific locations. SDNs, like meganucleases, zinc-finger nucleases, transcription activator-like effector nucleases (TALEN), and CRISPR-Cas nucleases, fall into three categories: SDN1, SDN2, and SDN3. SDN1 induces a random deletion, substitution, and/or insertion of base pairs (in a site-directed location), leading to loss-of-function mutations similar to natural occurrences. SDN2 systems can precisely modify a few bases at a DNA break using a repair template, resembling natural mutations achievable via conventional breeding. SDN3 incorporates longer DNA segments, like entire genes, for directed genetic modifications within the same species (Ahmad et al., 2021).

According to the revised draft of GFI #187 by the US Food and Drug Administration (FDA), titled 'Regulation of Intentionally Altered Genomic DNA in Animals,' Intentionally Altered Genomic DNA refers to intentional changes made to genomes using modern molecular technologies. These modifications involve deliberate changes in DNA sequences, which could be either random or targeted, including nucleotide insertions, substitutions, or deletions. These genetic alterations, combined with other methodologies, lead to specific modifications to an animal's genome (FDA, 2017).

The EU Commission has introduced a draft proposal for an amended regulation on New Genomic Techniques (NGTs) or gene editing methods used to induce targeted mutations (mutagenesis) in living organisms' genomes. The listed NGTs encompass various techniques such as cis-genesis and intra-genesis, zinc finger nuclease technology (broadly defined as site-directed nuclease technology), oligonucleotide-directed mutagenesis, RNA-dependent DNA methylation, grafting (on genetically modified rootstock), reverse breeding, agro-infiltration, and synthetic genomics. In the realm of NGT plants, Category 1 constitutes those exhibiting 'conventional-like' traits, mirroring genetic changes occurring spontaneously or through conventional breeding, encompassing random mutagenesis. Meanwhile, Category 2 encompasses all other NGT plants not meeting the 'conventional-like' criteria (EFSA et al., 2021; Dima et al., 2023)

In accordance with the UK genetic technology bill (UK, 2023), precision breeding encompasses various breeding technologies like gene editing, allowing for significantly more efficient and precise DNA editing compared to conventional breeding methods. These precision breeding technologies facilitate targeted genetic alterations, generating advantageous traits akin to those achievable

through traditional breeding and natural occurrences. This distinguishes it from genetic modification, which involves modern techniques inserting functional DNA from one species into another.

REGULATORY APPORACHES FOR GE

Regulations concerning genetically engineered or genome edited (GE) organisms, encompassing animals, exhibited global diversity (Maxmen, 2017; Lindberg et al., 2023).

North America

In the United States, oversight of GE products is divided among three agencies: the Food and Drug Administration (FDA), the Environmental Protection Agency (EPA), and the Department of Agriculture (USDA). Each agency held distinct responsibilities related to GE products. The FDA primarily supervised GE animals intended for consumption, conducting safety assessments and categorizing them as novel animal drugs within its Center for Veterinary Medicine (CVM). Meanwhile, the EPA's purview involved GE animals designed to produce non-food substances, such as pharmaceuticals. The USDA's oversight extended to GE animals within agriculture, encompassing animal welfare considerations and evaluating environmental impacts. The USDA regulations outline specific exemptions for plants with singular genetic modifications, including repairs from cellular DNA breaks, targeted single base-pair substitutions, and the introduction of genes already present in a plant's genetic pool. Since 2011, these regulations have allowed for exemptions for additional plants with modifications achievable through conventional breeding. Moreover, plants resembling those previously assessed and deemed low-risk by the USDA also qualify for exemption. The FDA clarified its regulatory scope by encompassing both rDNA technology and genome editing in animals through a revised draft Guidance for Industry #187. Canada's regulatory approach focuses on the final product's characteristics, regardless of its creation method. Just like traditional breeding and recombinant DNA techniques, gene editing can result in both novel and non-novel traits. Health Canada mandates pre-market safety assessments solely for gene-edited products identified to possess novel traits. Additionally, in 2022, Health Canada released a scientific opinion outlining regulations for gene-edited plant products. It emphasizes that novel food products derived from any breeding method presenting potential food safety risks will undergo a safety assessment following domestic guidance aligned with the Codex framework for biotechnology-derived food safety assessments (FAO, 2023).

South America

Resolution 176/2015 in Argentina established criteria for categorizing new breeding technique products, such as gene-edited organisms, as GMOs. Subsequently, Argentina labeled numerous gene-edited plant and animal lines developed for agriculture as non-GMO (Whelan et al., 2020). Meanwhile, Chile's Service for Agriculture and Livestock (SAG) clarified Resolution No. 1523/2001's applicability to new plant breeding techniques, adopting a case-by-case evaluation using a standardized form. This form collects specifics like species, variety/line, phenotype, and developer while also detailing the breeding process and technique characteristics. Additionally, it seeks information regarding prior releases and permits from other countries. Normative Resolution No. 16/2018 by Brazil's CTNBio establishes guidelines to determine if products developed via New Breeding Techniques (NBTs) should be classified as GMOs or non-GMOs. Developers need to provide detailed information about the original organism and the resulting product, including the methodologies used and molecular analysis. Non-GMO classification involves criteria like the absence of recombinant DNA/RNA, the presence of genetic elements obtainable through traditional breeding, induced mutations similar to established methods, and naturally occurring mutations. Certain techniques, like products from SDN1 or SDN2 mutations meeting RN16's criteria, are exempt from GMO classification, while transgene inserts through SDN3 mutations usually undergo a case-specific assessment and might be classified as GMOs. GMO-labeled products require compliance with biosafety rules and CTNBio's risk evaluation, while non-GMO products follow standard registration processes. Normative Resolution No. 16 applies universally to plants, animals, and microorganisms, covering both research and commercial stages (Rozas et al., 2022).

Japan and China

In Japan, government ministries such as the Ministry

of Environment (MoE), Ministry of Health, Labour, and Welfare (MHLW), and Ministry of Agriculture, Forestry and Fisheries (MAFF) issued guidelines to delineate the regulations concerning genome-edited products. MoE assesses these products based on the criteria for Living Modified Organisms (LMOs), taking into account factors like extracellularly processed nucleic acids and the integration of genetic material from compatible species. Meanwhile, MHLW excludes gene-edited food that poses risks similar to conventionally bred products from undergoing GMO safety assessments, emphasizing the absence of foreign DNA and specific alterations induced by site-directed enzymes (Kondo and Taguchi, 2022). In China, the Ministry of Agriculture and Rural Affairs (MARA) provided safety evaluation guidelines for genetically engineered plants used in agriculture. These guidelines focus on gene-edited plants without introduced external genes and offer distinct procedures based on risk levels. For plants with minimal food or environmental risk, the guidelines outline a simplified registration process compared to transgenic plants (USDA, 2022).

European Union and United Kingdom

The European Union's Court of Justice ruled that mutagenesis-modified organisms, irrespective of the method, are considered GMOs under Directive 2001/18/EC. Only those derived from established, extensively used mutagenesis techniques are exempt. Consequently, organisms from new methods like genome editing fall under GMO legislation. Commissioned by the Council of the European Union, a 2021 study reviewed new genomic techniques developed since the 2001 GMO legislation, encompassing gene editing. Following this study, the European Commission plans a policy initiative focusing on plants developed through targeted mutagenesis and cis-genesis, covering derived food and feed. Finally, the Commission proposed a regulation on new genomic techniques (NGTs) on July 5, 2023. It defines two categories of NGT-derived plants: those similar to naturally occurring or conventional plants and those with more intricate modifications. Each category will face distinct market entry requirements, tailored to their specific characteristics and risk assessments (Dima et al., 2023). In March 2023, the Genetic Technology (Precision Breeding) Act (2023) in the UK delineated that crop varieties or animal breeds developed through processes resembling natural genetic changes or traditional breeding are exempt from GMO regulation. This legislation introduces the concept of 'precision-bred organisms,' emphasizing gene-editing techniques that avoid introducing foreign DNA.

RISK ASSESSMENT IN GE ANIMALS

The analysis of whole-genome sequencing (WGS) revealed the presence of unintended DNA sequences, including DNA vectors utilized as templates during the gene editing process, within the hornlessness (polled) cattle. Despite these sequences not participating in protein synthesis, signifying a low risk, the potential for disease induction due to single nucleotide mutations underscores the need for a comprehensive risk assessment (Young et al., 2020).

The FDA, in March 2022, opted for enforcement discretion concerning the marketing of products, including food, from Acceligen Inc.'s "PRLRSLICK" genome-edited beef cattle and their offspring. This decision followed the determination that the intentional genetic alteration—specifically, prolactin receptor mutations—posed no safety concerns, marking the first low-risk determination for an IGA in animals used for food. Developers are typically required to obtain approval via new animal drug applications for IGAs; however, the FDA may waive this requirement on a case-by-case basis for low-risk edits. The data submitted to the FDA included detailed genomic information, such as whole-genome sequencing (WGS) and bioinformatics analysis of edited calves and their unedited parents. This analysis uncovered unintended alterations in the IGA-containing cattle. While the FDA confirmed these alterations, it concluded that they wouldn't impact protein expression or pose safety risks. Consequently, the FDA does not object to Acceligen marketing the IGA in PRLRSLICK cattle or their associated products for consumption. Additionally, it won't object to introducing these cattle into the food supply, limited to specific products and their progeny. The FDA intends to treat facilities engaging in standard agricultural practices for these cattle similarly to those without IGAs (FDA, 2021).

To date, Japan has seen the approval of three genome-edited foods, including tomatoes with heightened γ-aminobutyric acid (GABA) levels and two fish species, sea bream, and tiger pufferfish, which were authorized in 2021. These fish underwent genetic modifications using

Cas9 mRNA and gRNA, resulting in deletions in specific genes related to muscle development. Rigorous safety evaluations included whole-genome sequencing, PCR analysis, and allergenicity screenings, confirming the absence of foreign sequences, off-target mutations, and allergenic proteins. The regulatory discussions centered on assessing genetic changes in fish populations, concluding that specific genetic alterations at the target site in both alleles could be considered a single event (Kondo and Taguchi, 2022).

CURRENT REGULATORY FRAMEWORK OF LM ANIMAL IN KOREA

As of now, there have been no reported commercialized LM animals in South Korea; only experimental research has been announced. The current regulatory framework in the country defines terms related to genetic modification, modern biotechnology, and genetically modified organisms (GMOs) using criteria set in bio-safety guidelines. However, the existing regulations limit the definition to recombinant DNA, excluding comprehensive coverage of the latest new biotechnology advancements. Specifically, genetically modified animals are defined as those whose genetic material has been altered by recombinant DNA technology, encompassing modified gametes, embryos, and limited to cultured cells derived from fetal or adult organs. This implies that animals modified using advanced new biotechnology techniques like gene editing might fall outside the scope of genetically modified animals. Additionally, discrepancies exist between the regulations for livestock breeding management and breeding handling. For instance, while breeding management advocates segregating genetically modified animals from non-modified ones, breeding handling allows crossbreeding with non-modified animals, which might result in environmental release according to the regulation (RDA, 2017, 2020).

DISCUSSION

In South Korea, there's a growing trend toward relaxed regulations concerning induced mutation products due to advancements in gene-editing technologies. Recognizing its advanced standing in this field, the government acknowledges the necessity for enhancing its domestic regulatory framework. In 2019, a task force was assembled to refine biotechnology regulations, culminating in the submission of a bill after two years of extensive deliberation. One facet of the proposed amendments entails revising specific sections of the legislation governing the international transportation of genetically modified organisms (GMOs). Under amendment number 2116632, national authorities may exempt certain procedures if no foreign genes are present in either the organism or its final product. Given the limited familiarity with newly modified organisms, strategies involve acquiring genetic data and standard samples to devise detection techniques for monitoring purposes. Furthermore, the amendments include provisions enabling pertinent safety management authorities to obatin information and samples from developers. Recently proposed amendments primarily aim to enhance the disclosure of domestically imported unapproved GMOs, strengthen inspection protocols, reinforce governmental safety responsibilities, and conduct environmental impact assessments. Notably, these amendments prioritize managing incidents related to unapproved GMOs like imported zucchini, rather than specifically provide new regulations for risk assessment and safety concerning genome-edited animals and plants (Bill information system, Republic of Korea; https://likms.assembly.go.kr/bill/main.do). Furthermore, in many cases of producing livestock animals using genome editing technology (Park et al., 2019), somatic cell nuclear transfer (SCNT) is frequently employed. Hence, the assessment of safety for the commercialization of not only genome editing but also SCNT should be considered.

The current international regulatory trends concerning genome-edited plants and animals, using new biotechnological techniques, generally exempt these organisms from being categorized as GMO/LMO if they lack foreign genes and can occur through natural mutations or traditional breeding programs. Following comprehensive risk and safety assessments, these organisms can be released into the environment without specific labeling indicating gene editing. Consequently, the new regulatory frameworks extend their scope beyond domestically produced genetically edited animals to include assessments of risk and safety for imported agricultural products, germ cells, embryos, and stem cells. Efficient implementation of these regulations requires a meticulous analysis of LM/GE (Genetically Engineered/Edited) animal traits. This includes a detailed scrutiny of their intended applica-

tions (in food or medical domains), genome information, production methods, materials involved, and an assessment of the phenotypic impacts resulting from genome editing. Moreover, establishing a systematic and effective framework is crucial for evaluating the safety of animals, environmental/food safety, and the stability of genetic modifications in LM/GE animals.

Safety assessments of LM/GE animals involve examining unintended genetic impacts, biological consequences, and immunogenicity. Additionally, evaluations related to environmental/food safety should encompass assessments of animal contamination levels, environmental impacts, toxicity, allergenic potential, and methods for detecting genetic-level changes. Conducting such comprehensive evaluations and analyses is crucial for determining the stability of genotype and phenotype in genetically modified/edited animals.

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