Environmental Sciences Europe

REVIEW

Open Access

Existing and emerging mRNA vaccines and their environmental impact: a transdisciplinary assessment

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Abstract

mRNA vaccines have played a massive role during the COVID-19 pandemic and are now being developed for numerous other human and animal applications. Nevertheless, their potential ramifications on the environment lack scrutiny and regulation. On 14 July 2020, the EU decided to temporarily exclude the clinical trials with COVID-19 vaccines from prior environmental risk assessment. Even though billions of doses have been administered and large-scale agricultural and wildlife RNA applications are fast-tracked, there is no knowledge of their environmental impact via the dispersion of vaccine-derived material or their wastage. This knowledge gap is targeted here via a critical assessment of (1) the pharmacokinetic properties of these products; (2) their impact on the human microbiota; (3) novel risk factors exemplified by the human gut bacterium *Escherichia coli* resulting in pathogen evolution in the guts of wild animals, (4) findings on mRNA-LNP platforms that implicate extracellular vesicles (EVs) as superior carriers, and (5) potentials of exogenous regulatory RNAs. This analysis results in the first extrapolation of (a) the magnitude and likelihood of environmental risk as characterized by the FDA in 2015 for products that facilitate their action by transcription and/or translation of transferred genetic material or related processes, and (b) additional risks facilitated by the horizontal transfer of exogenous short RNAs. The arguments provided here establish the rationale for vaccine-derived bioactive material dispersed by EVs, impacted microbiota, and other exposed organisms to foster pathogen evolution, cross-species transfer of biological function, and driving widespread ecosystem disturbances. Evidence is emerging that vaccine-derived molecules, when ingested, could survive digestion and mediate gene expression regulation, host–parasite defense, immunity, and other responses in the consuming animals. Highlighting further unresolved questions, the comprehensive assessment provided here calls for open dialogue and more in-depth studies to get a clear picture in the EU and globally to most effectively gauge the environmental impact of existing and emerging human, livestock, and wildlife mRNA technologies or their potential as biological weapons or for other forms of misuse. Regulatory measures are urgently needed to mitigate potentially large-scale damage to public and ecosystem health as well as adverse societal, economic, and legal implications.

Keywords MRNA vaccines, Animal and zoonotic diseases, Environmental risk, Mobile genetic elements, Interkingdom communication and regulation, Extracellular vesicles

Background

The global application of mRNA vaccines during the COVID-19 pandemic has opened the floodgates for numerous novel RNA platforms for human and animal use. Yet, their environmental ramifications via their wastage and large-scale application including those in the open environment lack scrutiny and clear regulation.

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Specifically, on 14 July 2020, the Council of the EU provided a temporary derogation for clinical trials with vaccines against COVID-19 from the prior environmental risk assessment required in the EU legislation which applies for the duration of the COVID-19 pandemic or as long as COVID-19 is a public health emergency [15].

Meanwhile, concerns have been raised that vaccine material could be disseminated via extracellular vesicles (EVs), a topic that has been regarded as controversial. Indicators of "shedding" of the mRNA COVID-19 vaccines were first obtained by Bansal et al. [3] who demonstrated the seemingly prolonged presence of circulating exosomes containing vaccine-derived spike protein. These findings were disputed in a letter which then led Bansal and colleagues to backpedal some of their previous results, seemingly unaware of related pre-pandemic results [42] which they may have essentially replicated.

One can also envision that unrecognized environmental risks of the mRNA vaccines could be facilitated by a host of microorganisms that unintentionally get exposed to these drugs. However, even in this regard, literature results do not seem clear. Fundamentally, the gut microbiota is a very important and intriguing biosystem spanning the entire length of the digestive tract and playing important roles in health and disease. Already early in the pandemic, it became known that the gut microbiota is significantly affected by SARS-CoV-2 infection [4, 29, 86]. The converse, how COVID-19 vaccines affect the gut microbiome, seems to be less elucidated. Prior work [46] hypothesized that mRNA vaccines might be able to impair the human microbiome, based on the following rationale: (a) the wide dissemination and stable persistence of biologically active material (the targeted mRNA, unintended RNA/DNA byproducts from manufacturing, as well as various components of the lipid nanoparticles) derived from the vaccines. (b) The finding that mRNA vaccines, despite being intramuscularly injected, reach mucosal effector sites and trigger mucosal immunity to some extent; (c) adverse events post-jab reported globally which affect the GI system; (d) the involvement of exosomes as mediators of both intraspecies as well as interkingdom communication. However, the focus of Ref. [46] was primarily on the impact of mRNA technologies on humans. Their potential environmental ramifications were not further analyzed.

The demonstrated presence of vaccine-derived material throughout the body (summarized in Table 1) necessarily engenders substantial interactions with the gut microbiota. This is because these organisms are not only known to be intraluminal or attached to the linings of the gut. Some even occupy an intracellular or subepithelial intercellular residence and others enter the tissue fluid, the lymphatics, and even the bloodstream [35]. As basic factors of the bidirectional interaction between the injected material and the human/animal microbiota have not been sufficiently explained, the potential ramifications of their dispersion into the environment are even less known.

So far, 13.58 billion COVID-19 vaccine doses have been administered globally [61]. Furthermore, increasing previous open and closed vile wastage of vaccines, for the COVID-19 injections, early estimates [36] showed wastage rates up to 30%. In the fall of 2023, the E.U. alone discarded 215 million doses [41]. Despite the unprecedented scaling issues, policies to ensure sufficient deactivation of these new drugs when they are discarded, damaged, or destroyed, do not seem to have been implemented.

All this raises the concern of unknown ramifications, especially since novel mRNA technologies are rapidly being developed and rolled out, for various human conditions as well as for animals and wildlife. To help address this, this article aims to address the knowledge gap of the vaccine's potential ramifications on the environment by critically appraising (1) the information available in the literature about the persistence and distribution of vaccine-derived material in the human body, (2) pre-pandemic and emerging studies on mRNA-LNP platforms and the role of EVs as alternative carriers, by describing (3) potential mechanisms of how mRNA vaccines could impact the gut microbiome, but also (4) environmental organisms, and (5) the horizontal transfer of vaccine-derived regulatory RNAs and their potential biological functions.

In addition to the systemic dissemination of vaccine-derived biologically active material by EVs in the open environment, special focus will be placed on their fecal transmission via gut bacteria. This is expected to exacerbate demonstrated concerns of how mobile genetic elements derived from human pathogens such as *Escherichia coli*, in the already existing context of genetic or chemical pollution, may support their evolution in the guts of wild animals and lead to the danger of emergence of new pathogens and widespread ecosystem disturbances.

The article also assesses the question of whether ingestion of compounds derived from vaccinated animals could survive digestion. The potentially catastrophic implications are that vaccine-derived material may unwittingly (or deliberately) enter the food system at scale and influence gene expression regulation and other biological functions in humans and animals consuming those products.

Finally, the article closes with the first extrapolation of the magnitude and likelihood of environmental risk as characterized by the FDA in 2015 for products that facilitate their action by transcription and/or translation of

Table 1 Examples of studies demonstrating persistence and distribution of vaccine-derived components

Type of vaccine	Main findings	Source
Moderna's 2017 flu vaccine candidate	After IM administration in mice, mRNA is detectable in plasma, several organs/tissues, including the brain	Bahl et al. [1]—further analyzed in [46].
Synthetic mRNA-encoding hEPO protein	LNPs and EVs can disseminate exogenous hEPO mRNA in the blood and produce new protein in different organs and cells	Maugeri et al. [42]
COVID-19 mRNA-1273 vaccine	Systemic detection of full-length spike protein and S1 protein from the vaccine. S1 antigen was detected as early as day 1 post 1st dose; however, after dose 2, neither S1 nor spike was found, and both antigens remained undetectable through day 56.	Ogata et al. [53]
mRNA-1647 as a surrogate of mRNA-1273	A "relatively small fraction" of the IM-administered mRNA-1647 dose distributed to distant tissues in male rats.	EMA Covid-19 mRNA vaccine risk management—Moderna [18]
LNP-formulated modRNA as a surrogate of BNT162b2	Over 48 hours, distribution from the IM injection site to most tissues occurred with the greatest levels in plasma (tested in rodents).	EMA Assessment Report of Comirnaty [17]
COVID-19 BNT162b2 vaccine	Observed extended presence of vaccine spike protein and mRNA in vaccinee lymph node GCs for up to 2 months after vaccination. The amount of spike protein in the blood of vaccinees was up to thousands of times higher than that of spike protein in the blood after acute/severe Covid infection.	Röltgen et al. [60]
COVID-19 BNT162b2 vaccine	Vaccine-associated synthetic mRNA can be detected in systemic circulation for at least 2 weeks post-jab.	Fertig et al. [19]
COVID-19 BNT162b2 vaccine	Demonstrated very long-term persistence of the vaccine spike. Detected circulating exosomes expressing spike protein detected for at least four months after the second dose (see text for more).	Bansal et al. [3]
COVID-19 BNT162b2 or mRNA-1273 vaccines	Suggest that vaccine mRNA released into mammary cell cytosol can be recruited into developing EVs and secreted. Observed that the vaccine mRNA spreads systemically and is packaged into BM EVs. Examining 13 lactating women post-jab, detected the presence of very short vaccine mRNA sequences in BM EVs.	Hanna et al. [22, 23]
COVID-19 Pfizer/Moderna vaccines	Found that the COVID-19 vaccine mRNA can infiltrate the umbilical cord blood and penetrate the fetal–placental barrier. Demonstrated that the encoded spike protein can reach placentas and umbilical cords in pregnant women.	Hanna et al. [38]
COVID-19 mRNA vaccines	The post-mortem study found vaccine mRNA in the lymph nodes in the majority of patients dying within 30 following injection with the Moderna/Pfizer vaccine. Detected SARS-CoV-2 mRNA vaccines in the heart.	Krauson et al. [33]
COVID-19 Pfizer/Moderna vaccines	Detected spike protein deposition in the skin.	Magro et al. [39]

transferred genetic material or related processes, as well as a list of open questions (This work extends an earlier preprint published as [47]).

Basic unknowns

The textbook mechanism of RNA-based medical platforms hinges on either the expression of a targeted protein or the silencing of pathological genes through the delivery of some synthetically generated exogenous RNA to cells. In the context of mRNA vaccines, the targeted protein is, essentially, the spike protein of SARS-CoV-2. Yet, regardless of whether the goal is protein expression or gene silencing, the mRNA/siRNA must be taken up by the right cells and be able to escape the endosomes to be translocated into the cytosol. With mRNA COVID-19 vaccines, to facilitate this step, certain lipid nanoparticles (LNPs) have been used as delivery vehicles.

Before the pandemic, and as late as 2019, some of the main challenges and open questions with mRNA technologies that are relevant to their potential environmental impact were [16, 42, 46, 85]:

- The inherent instability of natural mRNA.
- The highly inflammatory nature of the synthetic RNA as well as their LNP carrier systems.
- The observation that only a small amount of the exogenous RNA carried by LNPs escapes the endosome to reach the cytosol even when a major proportion of LNPs is taken up by cells.
- Temperature instability, as well as tissue tropism questions of the LNP carrier, their poor mucosal immunity, and the fate of endocytosed LNPs (biodistribution, degradation mechanisms, their biological activity).
- Tissue localization and fate of the LNP-delivered modified mRNA (biodistribution, half-life, and persistence),
- The type and amount of the resulting protein product and its distribution and persistence.
- The type, amount, and fate of unintended genetic byproducts from the synthetic manufacturing processes (contaminants) and that of their expressed products.

Many of these questions remain incompletely resolved, despite the modifications done to uridine (aimed to stabilize the mRNA and allow the foreign mRNA to evade recognition/destruction by innate immune responses—the discovery of which led to the Nobel Prize in Physiology and Medicine in 2023 to Katalin Kariko and Drew Weissman) and various modifications to the mRNA (to further optimize protein production [17].

Contrary to previous expectations, the mRNA COVID-19 vaccines are now known to be disseminated throughout the body and largely resistant to natural degradation mechanisms (Table 1). Even though the existing studies reveal no complete picture, they do agree on the distribution and persistence [55] of significant amounts of vaccine-related material. While previous studies did not focus on this, these factors make the latter amendable to (further) transport by EVs or accessible to human microorganisms that live on or within human tissues and biofluids.

Also, rather than the expected superior translational capacity, such modified mRNA has been found to cause the protein-production machinery to stall during translation, leading to substantially increased misreading of mRNA and unintended protein products [48]. On the other hand, the amount of vaccine spike can be comparable to or significantly higher (up to thousands of times) than that found after natural infection [60]. Thus, while the prolonged persistence of the mRNA seems to be attributable to the pseudouridine, this does not fully explain the very high level of the vaccine spike (or even that of all the unintended protein byproducts).

Studies done before and during the pandemic [42, 49] have revealed the limited capacity of LNPs to undergo endosomal escape which, therefore, seems to hamper the use of LNPs as RNA delivery vehicles. Since it was found that only less than 2% of siRNA administered via LNPs escapes the endosomes, this raises the question about potential mechanisms that may explain the unexpectedly high protein yield from the mRNA COVID-19 vaccines. As further expounded in the following, some of these are expected to have substantial environmental ramifications as well.

The role of EVs

Some of the prime candidates for the transport of genetic material within and between organisms have been known to be facilitated by extracellular vesicles (EV) [63, 64]. EVs are broadly defined as membrane-bound vesicles released from cells. While originally believed to be mainly involved in the shedding of specific membrane functions and the discharge of cellular wastes, over the years, evidence has implicated EVs as an important means of intercellular communication via their ability to transfer proteins, mRNA/various microRNAs, and other bioactive molecules between cells.

Indicators of EV involvement in mRNA vaccines

Evidence for the tangible involvement of EVs in the dissemination of COVID-19 vaccine material was first given by Bansal et al. [3] who found a very long-term persistence of the vaccine spike of BNT162b2, and surprisingly, circulating exosomes expressing spike protein for at least four months after the second injection. Their foundational article evoked a letter that claimed various errors with the article. Responding to this letter, Bansal and colleagues conceded that they could not state that circulating exosomes generated after the first dose of vaccination persisted until 4 months after the second dose. However, an analysis [46] of the conjectured gaps brought forth in the letter shows that the discrepancies in the timing of the first appearance and persistence of the generated spike protein could be the result of some technical issues. An independent explanation for the kinetics discrepancy was very recently given in [55]. Indeed, Bansal et al., seemingly unaware of this, essentially reproduced the 2019 work by Maugeri and colleagues [42] that will prove to be indispensable in our context.

EVs suggested as a superior biological vehicle

In 2019, Maugeri et al. [42], aiming to find an alternative carrier to the LNPs, suggested that EVs could play this role. Published in Nature Communications, they demonstrated that EVs can indeed act as carriers of synthetic mRNA, able to take their cargo to distant organs and cells, where it was shown to be functional and produce the desired protein in mice. Key aspects of their model are:

- It may not be the LNPs alone that deliver mRNA to cells that express the targeted protein.
- Rather, part of the RNA delivery may be achieved via certain EVs secreted by cells that internalize the mRNA-LNP complexes.
- Specifically, part of the mRNA-LNP material that does not escape endosomes to reach the cytoplasm of cells can be packaged into EVs, secreted again from cells, and transported to new cells.
- This results in the dissemination of the synthetic mRNA to cells that may not be reached by the LNP carrier system (and also raises the possibility it is these latter mRNAs observed by Bansal and colleagues [3]).

The need to address some of the inherent problems with the LNPs has led to attempts whereby EVs are manipulated in numerous ways as an alternative carrier. In several experiments, exosomes loaded with an mRNA-encoding spike or nucleocapsid protein of SARS-CoV-2 triggered potent immune responses, including IgGs and secretory IgAs (reviewed in [49]), indicating they substantially disseminate within the mucosal compartment as well. Characteristics of EV-based vaccines, such as their increased stability even at room temperature, for more than a month when formulated as either inhalable dry powder or in lyophilized form, further raise the concern of environmental risk when exhaled.

The broad and stable dissemination of such carrier EVs seems to confirm their inherent characteristics envisioned by Maugeri et al. [42] and imply the following:

- EVs are potent carriers of exogenous RNAs irrespective of the way they have been administered.
- Their transportation of vaccine-RNAs effectively propagates biological activity much beyond the cells transfected by LNPs.
- For the mRNA vaccines, a large part of the delivery of the mRNA could be achieved by such EVs. This could help explain the resulting high spike protein expression in organs and cells distant from the injection site in the muscle (as indeed observed, see Table 1).
- In addition to bodily fluids, EVs loaded with vaccine material are likely effectively disseminated into the environment via exhalation as well.

Past concerns of environmental dissemination and "vaccine" classification

As indicated, EVs can substantially amplify the dissemination and expression of the synthetic mRNA. According to their inherent characteristics, this cannot be restricted to the transport of vaccine material within the body of vaccinees but also involves their environmental dispersion. It is not known which underlying mechanisms were envisioned, but it is now known that the potential for dissemination of vaccine-related material has long been taken seriously. Pfizer, in its clinical protocol [6] of its mRNA COVID-19 vaccine, was particularly concerned about environmental exposure by inhalation or skin contact, and such occurrences were supposed to be reported to Pfizer. Already in 2015, the FDA, in its 'Guidance for Industry' documents [80], was very concerned about such a "shedding" phenomenon—which only in recent years has become criticized and "fact-checked", raising massive concerns regarding their health implications for those who get in contact with vaccinated humans and animals as well as the larger environment. In the above guidance, the FDA defines shedding as a means of how a product is "excreted or released from the patient's body." Their guidance was written before the roll-out of mRNA vaccines but does apply to gene therapy products, which are defined, among others, as products "that mediate their effects by transcription and/or translation of transferred genetic material."

Aside from the fact that this precisely depicts the intended mechanism of mRNA vaccines, it is important to note that Moderna, in their official SEC filing in 2020 [79], explicitly stated that "mRNA is considered a gene therapy product by the FDA." Likewise, BioNTech founder Ugur Sahin previously noted that "[o]ne would expect the classification of an mRNA drug to be a biologic, gene therapy, or somatic cell therapy" [62], and as recognized by BioNTech in their official SEC filing [78], "In the United States, and in the European Union, mRNA therapies have been classified as gene therapy medicinal products." These products also fulfill several of the characteristics and criteria of human gene therapy products [46] as characterized by the FDA [82]. Furthermore, the nature of the COVID-19 vaccines as cell and/or gene therapy to promote related applications has been endorsed by the Head of Pharma at Bayer [84] as well as by George Church [11].

Finally, during the EU derogation of COVID-19 vaccines from environmental risk assessment [15], the

Council anticipated that those products would contain or consist of GMOs. For COVID-19, it seems feasible that the EU envisioned vector vaccines and traditional technologies. While there has been quite some debate about the classification of mRNA platforms as gene therapy products, especially from a clinical perspective, recently, some have argued they fulfill the legal definition of GMOs in most jurisdictions globally [20]. In sum, the lack of environmental impact studies is largely fostered by a regulatory gap. That the derogation should have been only temporary may have been overlooked by an inappropriate conception and classification of mRNA technologies.

Impact of COVID-19 vaccination on the human microbiota

The human microbiota is intimately linked with several COVID-related issues. However, strategies such as early antibiotic use or supplementation with probiotics have shown promising outcomes - apparently to prevent viral replication in the gut microbiome and/or to control toxicological production from the human microbiome [10, 25]. Regarding vaccination, it is also known that the gut microbiota can influence the immunogenicity and efficacy of COVID-19 vaccines [25, 52].

Several groups have investigated if the reverse is also true asking specifically whether vaccination could influence the microbiome (Table 2). Only one preprint study by Boston et al. [8] found no impact. Arguing that previous work had not assessed the short-term impacts of vaccination on the gut microbiome, they resorted to a different type of analysis than the others. This may also explain their disparate findings which may be consequent to (1) the focus on 'acute' effects (2–3 days post-injection) and that a reasonably short time thereafter (here called 'late' but is only 16–28 days after vaccination—much shorter than for [52] and [70]); (2) the way the analysis was performed: there was only a limited tracking of individual patients; rather, the DNA samples from the different cohorts (43 healthy control, 160 cancer, and 36 primary immunodeficient patient samples) pre-dose, 'acute', and 'late' were sequenced in bulk. In contrast, four other studies (Table 2) found that COVID-19 vaccination led to substantial changes in gut microbiota composition and function.

Mechanisms by which COVID-19 vaccines may affect the gut microbiota

Despite its importance, this topic has not received the attention it deserves, perhaps because of the mistaken conception that mRNA vaccine products are short-lived, localized in the injection site only, and not genetic therapies. However, a few potential mechanisms have been postulated in the literature, and a few additional ones seem feasible:

Alterations of the composition and functionality of the microbiota

- It is now well-established that the vaccines are widely distributed, evoking **systemic immune responses** and impacting intestinal epithelial architecture. In turn, these may alter the intestinal immune environment and thus affect the growth and survival of gut microbes [28]. For example, the release of various pro-inflammatory cytokines could impair gut permeability, disrupt gut microbiota equilibrium, and result in an increased abundance of opportunistic pathogens and a decreased abundance of commensal symbionts [87]. While this mechanism was postulated for SARS-CoV-2 infection, the same also applies to mRNA-LNP platforms, because of their inflammatory potential [21, 30, 42, 51, 59]. Specifically, the significant decline in *Actinobacteria* and *Firmicutes* abundances as observed by Ng and collaborators [52], they believe, could be explained by altered physiological functions and drastic inflammation consequent to the vaccine regimen.
- Another possible mechanism is via the inherent **interplay between the systemic and mucosal systems** [46]. Thus it seems feasible that the vaccines induce local immune responses in the gut-associated lymphoid tissue (GALT) such as the intestinal Peyer's patches and thereby impact immune responses and their interrelationship with the gut microbiota [14]. Indeed, the human epithelial mucosa, human subepithelial lymphatic tissue, and the bacteria of the human microbiome seem to be intrinsically interconnected [9].
- It is also possible that the vaccines could **affect the expression and function of ACE2 receptors in the intestinal epithelium** (e.g., via the produced spike protein [46]). As is well known, these receptors are key in SARS-CoV-2 entry. But they play additional roles as well. Specifically, the relationship between gut microbiota and intestinal ACE2 expression has been intensely studied [87], also related to COVID-19 disease outcome, and it is now clear that ACE2 receptors are involved in maintaining intestinal homeostasis and balancing the microbiota.

While the above has, more or less at least, been discussed in the literature, it does not seem that some other possible mechanisms have been considered which

Table 2 Impact of COVID-19 vaccination on gut microbiota and function

Type of vaccine	Study features and time frame	Findings	References
Most participants received the BNT162b2 Pfizer vaccine	Longitudinal study to compare the three cohorts: 'pre-dose' (before vaccination), 'acute' (2-3 days after vaccination), or 'late' (16–28 days after vaccination) for each vaccine dose	• The gut microbiome remains remarkably stable post-vaccination. • The surprising stability of the gut microbiota post-vaccination is irrespective of the diverse immune status, vaccine response, and microbial composition of the participants. • Extends to all levels measured, including diversity, phylum, species, and functional capacity.	[8]
The inactivated SARS-CoV-2 vaccine candidate BBIBP-CorV	Each participant was examined at baseline (two weeks prior to injection) and two weeks after vaccination (for 2 doses)	Vaccination elicited substantial modulations on gut microbial composition and functions.	[31]
The inactivated COVID-19 vaccine BBIBP-CorV	For each participant, contrasted stool samples collected at baseline (day 0) and on day 14 after the second injection (day 42)	• Observed a substantial difference in the microbiome composition between pre- and post-vaccination. • Identified a significantly altered microbiome functional profile between day 0 and 14 days post-second jab. • The vaccine-induced alterations of the functional pathways could also be associated with vaccine response.	[70]
The mRNA COVID-19 vaccine (BNT162b2) and the inactivated COVID-19 vaccine (CoronaVac)	A prospective, observational study to contrast individual stool samples at baseline (within 3 days of the first dose) and 1 month after delivering two doses.	• Revealed a significant change in the gut microbiome composition and function with no notable difference between the two vaccine groups at 1 month post second dose compared with baseline samples. • For both vaccines, a large number of species were diminished. • Despite the observed substantial loss in biodiversity, none of the participants reported significant dietary changes during the study period.	[52]
Different types of vaccines/boosters (including mRNA vaccines)	Investigated more generally fecal microbial features and pandemic-related mental health	• Both COVID-19 infection and vaccination significantly changed the overall gut microbial makeup. • However, infection and vaccination were associated with distinct relative taxonomic abundance profiles.	[40]

may be even more serious. These involve the potential of the microbiota, or their functionalities, to be directly altered by the vaccines.

Influences that may lead to microbial alterations

It is surprising that although mRNA vaccines and their products can persist for months in the body and that mRNA-containing vehicles can cross the blood–brain barrier, there is no in-depth research on how they may modulate the human microbiome or other microorganisms. Several aspects are worth mentioning.

Via EVs

EVs such as exosomes also play a substantial role in host–pathogen interactions [63]. Significant quantities of EVs have been found in mammalian feces which are derived not only from the host but also from bacteria or fungi likely originating from the intestinal epithelium. Now, as detailed above, EVs can transport various vaccine-derived materials including various RNAs and DNA–RNA hybrids. By facilitating the horizontal transfer of bioactive molecules such as proteins and polynucleotides, they can induce both epigenetic and genetic changes, and this likely includes the human microbiota as well.

Via circulating vaccine-derived components

Previous work [46] has devoted quite some attention to the possibility (and consequences on vaccinees) of microbes being genetically adulterated by the genetic injections. This same concern has been articulated elsewhere [8, 9]. Boston and colleagues [8] in their above-mentioned preprint about the vaccine impact on the gut microbiome, admitted that they had only observed changes at the taxonomic level and related to functional capacity. But, they said, they could not "rule out genetic changes at mutational levels that may alter the microbiota function." This concern seems reasonable, as further specified next:

Involving RNAs: While ideally, mRNA vaccines contain the intended mRNA and are purified from unintended byproducts, both the history and practical experience of mRNA technologies have confirmed the presence of aberrant/fragmented RNAs in these shots, including dsRNAs and various short RNAs [46, 74]. These RNA species, while inherently much more stable than mRNA, are naturally prone to integration by human microorganisms [26]. This effect is expected to be drastically enhanced by the increased persistence of the vaccine-derived RNAs via their synthetic modifications and also, as the lipid-stabilized RNA complexes diffuse throughout the body.

Involving DNA: The manufacturing of the synthetic mRNA for the mRNA vaccines is done in vitro using a DNA plasmid as a template. Historically, purification methods to eliminate unintended byproducts have proven challenging [46]. Further complicating this issue, both Pfizer and Moderna, for their COVID-19 vaccines, resorted to faster and cheaper processes to scale up production [7]. Rather than using a PCR-generated DNA template, the DNA was cloned into a bacterial plasmid vector for amplification in *E. coli*, further introducing unwanted genetic material into their products. However, the DNA from the expression plasmids used during manufacturing, while digested during purification, ended up contaminating the vaccine product in the form of numerous small DNA fragments. First reported by Kevin McKernan and collaborators [43], the high level of DNA contamination has independently been verified by several laboratories and acknowledged by the FDA, the EMA, as well as Health Canada [32, 71, 75]. Even though these agencies maintain that there is no concern for human health, additional quality concerns have arisen that nobody had predicted [48]. The ramifications of the contaminants could be disastrous and potentially irreversible [73].

In addition to the much-debated genotoxicity risk, the DNA fragments, also due to their large number, could directly impact the human microbiome. Notably, McKernan and colleagues have detected the presence of billions to hundreds of billions of DNA molecules per dose in these COVID-19 vaccines. It also appears that the DNA present in the vaccines is protected by encapsulation in the LNPs which greatly facilitates the transfection potential of eukaryotic cells and makes the DNA even more resistant to natural degradation. While bacteria frequently integrate free nucleic acids, considering they are also able to take up different EVs, it seems unlikely they will not take up DNA-LNP complexes as well. Unfortunately, the ramifications of this have not been determined.

mRNA vaccines and their potential risks on the environment

While certainly incomplete, several risk pathways and environmental hazards involving compounds from synthetic injections can be envisioned.

mRNA vaccines getting discarded but insufficiently degraded

The dissemination of DNA and xenogenic elements across waterways and the spread of genetically modified organisms (GMOs), antimicrobial resistance (AMR), and pathogens has raised increased concern, especially as new gene-editing tools, such as do-it-yourself (DIY) CRISPR-Cas kits have become deployable at the kitchen table and it became known that many of the widely used

sterilization methods are surprisingly inadequate [12]. Likewise, vaccine wastage, which happens when they are discarded, lost, damaged, or destroyed, has turned into a substantial global issue [83].

As stated, existing policies and regulations do not characterize mRNA vaccines as containing biologically active material or gene therapies. As a result, ramifications from the wastage of mRNA vaccines have not received much attention:

- In the fall of 2023, the E.U. discarded 215 million doses of COVID-19 vaccines. However, no details about this process were provided. It is not clear if they were regarded as chemical waste products and, therefore, carefully incinerated or if they were simply disposed of in landfills, as Politico indicates [41].
- However, the prevailing view that the contents of these products are all-natural and quickly degraded raises concern that these products may be inappropriately discarded, especially at scale.
- As a result, it seems unlikely that all of their active ingredients, including their synthetically stabilized genetic material and unanticipated contaminants, will be sufficiently degraded, leading to environmental risks that have not been characterized.

The danger of disseminating antibiotic resistance genes

Of great concern with the mRNA vaccine DNA contamination is that some of the impurities consist of entire Kanamycin/Neomycin resistance genes, even with their promoter [43]. When those genes integrate into the human microbiome, this will make them resistant to those antibiotics. In turn, when these microbiota spread into the environment, this could have additional far-reaching implications.

In 2015, in their guidance for industry [81], emphasizing the danger that some gene therapy-based products retain a level of detectable biological activity, the FDA warned:

"For example, a super-coiled plasmid may still retain the ability to transfer genetic material (such as antibiotic resistance genes) to other bacteria even after limited degradation. Therefore, the persistence of antibiotic resistance genes in the environment should be considered. Although the likelihood of this event may be low..., the impact of the event may be significant if, after a rare transfer event, the antibiotic resistance gene could spread to environmental organisms and potentially compromise existing treatments."

With mRNA vaccines, the potential for this risk is substantial for several reasons:

- In contrast to the situation analyzed by the FDA, namely that antibiotic resistance genes only enter the environment when the gene therapies or recombinant viral/microbial products have undergone some deliberate, albeit incomplete, degradation process, contaminants from mRNA vaccines create an unprecedented scaling problem.
- The work by McKernan, Buckhaults, and others shows that each of the tested vials of both the monovalent and bivalent vaccines has billions of pieces of plasmid DNA encapsulated in lipid nanoparticles, including antibiotic resistant genes.
- Disseminated by EVs and/or bacteria, a significant number of them may reach the groundwater and/or mingle with environmental organisms, evoking the danger of antibiotic resistance propagation in the open environment.

Affecting genetic changes in susceptible organisms

As detailed above, EVs can transport vaccine-derived materials, including RNAs, DNAs, and DNA–RNA hybrids. By facilitating the horizontal transfer of bioactive molecules such as proteins and polynucleotides, they can induce not only epigenetic, but also genetic changes in exposed organisms. Bacteria are especially adept in taking up genetic compounds and incorporating them into their gene regulation, and there is no reason to believe this cannot apply to those derived from mRNA technologies. Importantly, if human microbiota, when adulterated by the injections, get dispersed, they could widely disseminate mobile genetic elements and complement existing risks, such as the **novel genetic combinations happening in the wild**.

A recent article in iScience [34] reveals some sobering findings of bacterial ecology and evolution in wild animal populations, exemplified by *E. coli*. Emerging evidence suggests that human populations have heavily influenced the gut microbiome of wild animals. Specifically, by sequencing whole genomes of 145 *E. coli* isolates from 55 wild and 13 domestic animal fecal samples in the California Bay Area, Lagerstrom and colleagues found:

- *E. coli* from this wild animal community harbor substantial pan-genomic diversity that is largely undefined. This large diversity is attributed in great part to horizontal gene transfer between strains and other bacterial species. Individuals carry multiple strains simultaneously, facilitating within and between host mixing.
- Extensive environmental pollution by antibiotics has created unprecedented selective pressures on bacte-

ria and contributes to the rapid and global spread of resistance. In addition, the mixing of human and wild bacteria in the environment is a massive but underappreciated contributor to the emergence of new pathogens.
- The vertebrae gut can serve as a "melting pot" of novel genetic combinations. In the context of antibiotics, chemical pollution, and mobile genetic elements, some pathogens may further evolve in the guts of wild animals.
- The large diversity of *E. coli* in wild animal hosts demonstratively reflects human impact. Among the tested animal community, host-associated *E. coli* carried a vast repertoire of virulence-associated genes; nearly half were known pathogens to humans. Clinically relevant antimicrobial resistance was found on mobile genetic elements.

The analogous mechanisms could allow vaccine-adulterated microbiota to infiltrate global water systems, leading to deleterious downstream processes. Thus, vaccine-derived material could affect **genetic adulterations in many different organisms**. This first concerns those that have RNA, or a mix of RNA and DNA, genomes. But it also applies to those whose genome is described as composed of DNA. This is because many such organisms (including eukaryotes) have stable RNA elements in their genomes that can be changed by RNAs. Moreover, RNA can also affect changes to DNA. Specifically, RNA-based formulations can be heritable, modify genes or other genetic material, be replicated by reverse transcription, and alter traits and heritable traits [26]. Even eukaryotes have stable RNA elements in their genomes that could be adulterated by exogenous RNAs. Although some of the genetic changes may be regarded as small, their effect can only be known by considering each occasion by itself, as even the smallest changes have been shown to completely alter key features of the organism, including its toxicity [27, 46].

Reference [46] previously conjectured that these effects could analogously apply to COVID-19 vaccines [46], possibly via human microbiota. However, nothing is known about the ramifications on the larger environment, including those involving large-scale mRNA vaccination of livestock and wildlife.

Vaccine-derived EVs likely influence the entire environment

EVs have been best characterized for their intercellular communication. However, they are not bound to intraspecies interactions but are also capable of interkingdom communication between humans, animals, plants, and microbes [64]. It has been speculated [46] that they may thereby significantly contribute to underappreciated ramifications of mRNA vaccines to the larger environment via EVs containing vaccine or vaccine-derived material. This is supported by the findings that

- In mammals, EVs can be derived and shed from tumors, body fluids, and cells [64]. The human microbiota can also take up (and transfer) EVs, including exosomes, microvesicles, and other types of vesicles.
- Common interkingdom crosstalk between animals and plants consists of the shedding of EVs from host microbiota or intestinal cells and plant exosomes. In turn, plant-derived EVs can be disseminated via fruits, seeds, and pollen and thereby impact bacteria, fungi, parasites, insects, and animals.
- EVs can carry mRNAs or short RNAs and have them expressed/act as regulatory RNA in the recipient species (for more, see below). They can support defense mechanisms and can themselves be pathogenic: for example, bacterial EVs can provoke severe immune responses and signs of septic shock, even without the presence of living bacteria [54, 66].
- As EVs are known to participate in numerous physiological or regenerative processes as well as infection and disease, vaccine-derived EVs can virtually influence the entire environment. The extent to which this can disrupt harmony or introduce adverse effects is difficult to estimate—but it is unlikely that they will not have any impact at all.

The aforementioned findings on the EVs indicate their potential as carriers of vaccine-derived material and their transmission via various bodily fluids, breath, and microbiota, therefore creating a complex network interacting with the already existing interkingdom communication system and likely engendering numerous effects.

Horizontal transfer of vaccine-derived regulatory RNAs and their potential biological functions

The potential of vaccine-RNA-mediated crosstalk between species has not received sufficient attention [46]. Notably, there is increasing evidence that short RNAs (miRNAs and siRNAs) can be transferred between cells, tissues, and even across species. Short RNAs derived from longer dsRNAs are at the heart of RNA interference (RNAi), a process that results in repression, translational inhibition (and sometimes also upregulation) of target genes through partial complementarities with various response elements of the target mRNAs. While the nomenclature of short regulatory RNAs is expansive, no clear distinction can be made in the kinds of silencing that many of these dsRNAs cause [26]. Importantly, dsRNA fragments are known contaminants of mRNA

vaccine production. Analyzing the underlying mechanisms of RNAi, the concern has previously been raised that those RNAs may act as regulatory RNAs as well [46]. However, potential consequences on the larger environment have not been elucidated.

Short RNAs such as miRNAs exhibit ultra-stability in the extracellular milieu that largely has been attributed to them being packaged into exosomes. Their **EV-based transport** can enable their broad dissemination across species, as discussed above. When **taken up** by recipient organisms in the open environment, in those, the transferred RNAs may exhibit biological functions that remain incompletely characterized. Three main mechanisms can be envisioned that also seem relevant when the EV cargo involves vaccine-derived RNAs:

- **Via direct interaction of EVs**: For example, exosomes secreted by plants have been shown to directly interact with fungal, mammalian, and bacterial cells [64].
- **Symbiotic interactions and those shaping the host–parasite arms race**: EVs also play a substantial role in interactions between a host and its symbiotic or invading organisms [63]. Indeed, the majority of examples of cross-species transfer of short regulatory RNAs come from interactions between host and invader. Numerous examples are known of the mutual exchange between hosts and their pathogens, parasites, and symbionts, both as a defense and a means to hijack this defense [89].
- One of the most troubling aspects of **exogenous RNAs** is that they **may survive digestion and thereby remain active in recipient organisms**. Already in 2012, Zhang and colleagues [88] reported that plant short RNAs ingested from food pass through the GI tract, enter into the bloodstream, accumulate in tissues, and can regulate transcripts in consuming animals. Surprisingly, a specific plant miRNA from ingested rice even appeared to modulate the expression of a receptor involved in LDL removal from mouse plasma. Subsequent studies investigating interkingdom transfer of animal and plant miRNAs have reported contradictory or negative results [89]. However, a detailed follow-up analysis [89] validated the substantial role of food/feed-mediated transfer of regulatory RNAs across kingdoms. They demonstrated the accumulation and function of numerous dietary miRNAs in specific animal tissues, which was validated by three different techniques (high-throughput sequencing utilizing high sequencing reads only, qRT-PCR assays with various controls, and Northern blots). In addition, after reviewing a number of independent studies, they found strong evidence that ingested short RNAs can indeed have a functional impact on consuming organisms.

Previous studies of the horizontal transfer of mobile regulatory RNAs have focused on their potential effects on humans and animals. Substantially compounding this existing concern, vaccine-derived short RNAs with regulatory function, disseminated throughout the ecosystem, could literally impact all organisms. Mammals do not have a process similar to *Caenorhabditis elegans* that allows the amplification of small amounts of environmentally derived RNAs [89]. However, there may be quite many organisms where such amplification pathways could trigger an extensive response. In sum, the synthetic modification of the vaccine-derived RNAs, combined with self-amplifying RNAs or the sheer scale of environmental applications of RNA technologies, could also result in sufficient amounts of stable RNAs to be transferred to have a tangible biological effect on organisms in the open environment.

As further studies to evaluate the potential of vaccine-derived transfer of mobile RNAs and their biological function are urgently required, the following potential aspects seem mostly troubling:

- **Impacting food and feed:** The above raises the concern that vaccine-derived material, disseminated from vaccinated humans or animals, may enter the food system. If such exogenous RNAs from food/feed can analogously regulate the expression of genes in mammals, then this could have catastrophic implications on the food supply of entire regions or even globally. This danger would be greatly amplified by mRNA technologies that are intentionally ingested.
- **Modulating the immune system:** Perhaps one of the most delicate aspects of exosomal short RNAs was described in the context of raw cow milk's consumption in the first year of life [44]. This study suggests that milk transfers microRNAs that are important for the development of the immune system. They may induce pivotal immunoregulatory and epigenetic modifications required for T cell maturation and may explain the atopy-protective effects some have observed with raw cow's milk consumption. The authors believe that this concept of supporting the development of the immune system via milk-mediated microRNas should be pursued by the addition of appropriate microRNA-enriched exosomes to infant formulas.
- **Vaccination during pregnancy:** If some exosomal microRNAs survive digestion (as indicated above), this could analogously have drastic effects on the

babies of mRNA-vaccinated mothers. Even though there are currently no large studies further examining this, the findings by Hanna and collaborators [23] of the presence of vaccine-derived short RNAs in the breast milk of vaccinated women show these concerns must be taken seriously.

- **Influencing host–pathogen interactions:** According to Zhou and colleagues [89], mobile RNAs should be regarded as potent weapons in the host–parasite arms race. On the one hand, both animals and plants can transfer short regulatory RNAs to interacting pathogenic and parasitic organisms to silence their transcripts and suppress their growth. Interestingly, the movement of such molecules has also been reported in the opposite direction, from invader to host, to hijack host defense mechanisms. This raises the concern that vaccine-derived short RNAs could enhance the resistance of certain pests against certain defense processes or, conversely, foster the genesis of more pathogenic microorganisms.
- **Other concerns** miRNAs are fundamental regulators of post-transcriptional programs. However, most of their delicate roles are still poorly understood, both in humans and much more so regarding their regulatory mechanisms across the entire ecosystem. Additionally, the immune systems of many of the animals planned to be injected with mRNA vaccines are drastically different. As RNAi pathways are found in nearly all eukaryotes, but with notable differences between animals and plants [26], the impact of exposure to vaccine-derived short RNAs in the open environment will be complex and may be difficult to classify. In general, the outcome of RNAi is largely determined by the binding strength of the guide strand (obtained from the dsRNA molecule) and the target RNA, but partial complementarity is known to engender regulatory mechanisms as well. Strikingly, the COVID-19 vaccine RNA shares extensive homologous features with human RNAs, [69] that can be problematic for several clinical reasons [46]. A comprehensive analysis of the similarities of vaccine-derived RNA fragments with those of environmental organisms seems insurmountable. Regardless, the sheer scope of these interactions predicts substantial potential for their regulatory influences throughout the ecosystem. The implication is that if these RNAs get transferred to susceptible species and accumulate in large enough quantities, they could, in analogy to traditional regulatory RNAs [89], influence gene expression regulation, environmental sensing, crosstalk between species, immune responses, plant nutrient composition, and other biological functions.

Emerging applications

mRNA vaccines either have been or are now being developed for numerous other applications for humans (e.g., for cancer, influenza, respiratory syncytial virus (RSV), Lyme, Marburg virus, Zika fever, HIV, malaria, and others) and a variety of different animal species [37]. Already in 2012, the Iowa-based vaccine producer Harrisvaccines announced the US Department of Agriculture (USDA) granted the first license for an RNA technology vaccine to be used in livestock, in particular for swine influenza virus (SIV) H3N2 [57] in 2014, it received conditional licensure of its Porcine Epidemic Diarrhea Virus (PEDv) Vaccine [5]; its SirraVax Platform can be extended to "any disease" as only an electronic gene sequence from a particular virus or pathogen is needed [24]. Furthermore, the veterinary mRNA particle type "vaccine" Sequivity is authorized for pigs in the USA [45]. Even though veterinary RNA vaccines have raised concern [50], because of the widespread use of the COVID-19 vaccines, it is expected that its utilization will be accelerated; for example, in Australia, it has been fast-tracked [67].

Not only will the widespread use in animals vastly boost the scale of interactions and amplify the effects envisioned above, in addition, emerging delivery platforms and routes of administration, such as via coated feed or through food [65], intranasal [56], and aerosolized/inhalable [68], would yet again lead to a substantial scaling increase of exposure pathways or engender additional environmental risks, for example via incompletely understood direct/indirect effects in organisms with vastly different immune systems including shrimp [58]. The emerging applications, also involving novel platforms utilizing self-amplifying mRNA vaccines [76] and circulatory RNAs [2], may enhance undesirable biological activity as well as underappreciated microRNA-based regulatory effects. Likewise, novel chemical modifications and enhanced thermostability [77] under investigation for various animals may make products or activities derived thereof even more stable and transmissible, with environmental effects that have not been adequately characterized.

Open questions and conclusion

Information provided in the literature [26, 34, 42, 49, 64] has revealed how little we know about the fate of synthetic genetic material, their interactions with natural life forms, or even the evolution of model pathogen organisms such as *E. coli*. The above analysis indicates the large-scale impact of existing and emerging mRNA vaccination programs on humans, livestock, and wildlife. This potential of dispensing biologically active material via microorganisms, EVs, or others will further exacerbate the Anthropocene and its ecological crisis in ways

Table 3 Relevant challenges and open questions

Type	Difficulty/open question
Analytic procedures	Detection methods of vaccine material and derived components are insufficient and often reveal conflicting results.
The total gene pool	Even for the model organism *E. coli*, only about 6% of the pan-genome, i.e., the total gene pool encompassed by all *E. coli*, has been isolated [34]. Due to horizontal gene transfer and exchange of mobile genetic elements, it could be nearly infinite.
Genetic adulterations happening in the environment	A lack of complete understanding of causes and biological effects of environmental mutations [26, 27].
Genotype–phenotype associations	Our understanding of genotype–phenotype associations is drastically incomplete. For example [34], even for the well-studied laboratory model organism, *E. coli K-12*, 35% of its genes are still lacking experimental evidence of function and another 5% are only known as pseudo- or phantom genes.
Determinants of pathogenicity	What makes an organism pathogenic is insufficiently known. For example, it has only recently been that suggested microbial pathogenesis may be a "coincidental" by-product of their genomic plasticity by enabling adaptation to a broad range of environments [34].
Chemical modifications of ribonucleotides	May have unintended regulatory properties, lead to new traits or even change the genome of organisms in an inheritable manner [26].
Connectivity	Very limited knowledge about microbes, microRNAs, and EVs, to affect intra- and interspecies, or even interkingdom communication.
Regulatory RNAs	Incomplete knowledge of small RNAs being carried across species and able to regulate biological activities in recipient organisms.
Influences beyond genetic determinants	Insufficient understanding of temporary/inheritable epigenetic influences and propagation of traits, e.g., via abnormally folded proteins (such as prions which have also been implicated with mRNA vaccines[50].
Genotoxicity of synthetic RNAs	Incomplete comprehension of genetic changes induced by RNA platforms. While this is heavily debated, especially related to their DNA contaminants, processes via various reverse transcription mechanisms and/or the involvement of the human microbiome and off-target species, have not been fully accounted for [46].
Pharmacodynamics of synthetic RNAs	Clearance mechanisms and elimination half-time of exogenous synthetic RNA in different tissues and animal species are insufficiently known.
mRNA-LNPs as biological activities	Incomplete understanding of both the mRNA and the LNPs as biological activities [13], including their underlying mechanisms, the full scope of effects, and manners in which these can be disseminated in the open environment.
Inheritability of traits	Incomplete comprehension of how RNA-vaccine-induced activities and traits are inheritable. For example, Qin and colleagues [59] found that the mRNA-LNP vaccine platform induces long-term immunological changes that can affect adaptive immune responses and heterologous protection against infections, and also alter innate immune fitness. Notably, mice pre-exposed to the mRNA-LNP platform were shown to pass down the acquired immune traits to their offspring. The mechanisms responsible for these effects are unknown.
Durability of effects	Incomplete understanding "immediate", "short-term" and "long-term" effects, especially when considered for all potentially exposed organisms and life-forms.

that previously did not seem to have been envisioned (bold-faced features are items the FDA says should be carefully examined for their environmental risk for products that mediate their effects by translation of transferred genetic material and related others [81]):

- The dispersal of vaccine-derived **biologically active material** via EVs and microbiota may evoke epigenetic and **genetic alterations** in environmental organisms, raising genotoxicity concerns across entire ecosystems that may also be inherited.
- The new traits or **metabolites** of vaccine-adulterated microbiota or those of the environmentally impacted organisms can disrupt the natural balance, increase their evolutionary **advantage**, **tropism, or host range**, or support the **colonization** of **novel pathogenic organisms** or otherwise be **toxic** to susceptible organisms.
- As a consequence of the synthetic modifications done to the RNA, and further complemented by the LNPs, to enhance their **stability**, the vaccine-derived biological activity is expected to be **persistent** and **stably** propagated.
- DNA contamination found in both the monovalent and the bivalent vaccines include plasmids encoding **antimicrobial resistance genes** and may further the **development of new antibiotic resistant pathogens**.

Fig. 1 Main contributors and challenges that call for urgent discussions and policy regulation of large-scale mRNA applications

Specifically, mRNA vaccines may substantially contribute to the evolution and emergence of novel "zoonotic" pathogens created by the mixing of human and wild bacteria and that of natural and synthetic genetic material in the environment. However, the horizontal transfer of exogenous vaccine-derived genetic material has not been rigorously analyzed. This adds to the concern for environmental genetic adulterations of exposed organisms and further suggests that some of such fragments may achieve regulatory function in different species. In particular, the horizontal transfer of mobile small RNAs has widely been underappreciated, misunderstood, and inappropriately excluded from regulation [26], even in the context of gene-edited insecticidal RNAi plants [72]

Evidence has been emerging that vaccine compounds can be environmentally transferred, ingested by exposed organisms, and survive digestion. This troubling aspect raises serious concerns in light of vaccine material found in the breast milk of vaccinated women and, more generally, for the planned large-scale utilization of RNA vaccination of livestock and animals. If vaccine-derived molecules ingested from food/feed indeed can pass through the GI tract, be transferred to the blood, and accumulate in tissues, they may mediate interkingdom gene expression regulation, host–parasite defense, and many other important biological functions.

It is not clear when and how potential adverse effects will be identified and characterized as many established and novel challenges and questions relevant in this context have not been resolved (summarized in Table 3). For example, the surprising findings by Lagerstrom et al. [34] that the use of antibiotics has shaped environmental pathogen evolution and the striking potential of horizontal transfer of exogenous regulatory RNAs across species when ingested or transferred via EVs indicates an underappreciated "scaling" [27] of unintended and adverse effects via the dissemination of synthetic genetic compounds. However, previously this has not included products whose genetic makeup has been deliberately stabilized to resist natural degradation processes such

as those employed for existing and emerging mRNA applications.

The main contributors and risks described above (summarized in Fig. 1) such as the potential development of new pathogens, contribution to environmental toxins or (epi)genetic adulterations in the open environment, or even the large-scale corruption of food and feed, will not only imperil public and ecosystem health. They also raise urgent questions related to organic/non-GMO regulations, indigenous rights, and whether their aftermath could be characterized as a "global public health threat" for humans or animals evoking potential emergency restrictive measures.

Other types of problems also call for urgent attention. For example, if bacteria or other organisms take up short gene fragments derived from such patented products and integrate them into their gene regulation, what are the legal or intellectual property ramifications, e.g., in terms of ownership rights or liability in case of harm? And finally, given the lack of regulation and oversight, what are the potentials of misusing mRNA technologies for the development of biological weapons or malignant purposes?

In light of plans to monumentally increase the application and scale of mRNA technologies to livestock, fish, and wild animals, via circulatory RNAs, self-amplifying, self-spreading or other novel platforms, and administration routes such as via food/feed or aerosolized, the risks and concerns described here call for open discussion, in-depth studies, and urgent regulatory measures to prevent potentially irreversible large-scale and far-reaching ramifications.

Acknowledgments
I would like to thank the reviewers for their time and effort. I appreciate the valuable comments and suggestions that helped improve the quality of the manuscript.

Author contributions
The author is responsible for the conception of the study, literature review and interpretation, drafting and writing the article, and has approved it for submission. The author is an experienced independent transdisciplinary researcher with a Masters in Mathematics/Statistics with (highest distinction, University of Klagenfurt, Austria), a PhD in Mathematics (summa cum laude, Univ. of Klagenfurt), 'Habilitation' in Mathematics, Data Security and Cryptography (Univ. of Klagenfurt), and a PhD in Biomedical Sciences (GPA of 4.0, University of Wyoming, USA). She has been a member of The European Network of Scientists for Social and Environmental Responsibility (ENSSER) since 2020, is also the author of "Challenges and Opportunities of mRNA Vaccines Against SARS-CoV-2—A Multidisciplinary Perspective" Springer 2023, and has been a volunteer contributor to several open-ended online Forums of the Convention on Biological Diversity.

Funding
There is no funding source associated with this research. The research is a product of independent work by the author.

Availability of data and materials
Not applicable.

Declarations

Ethics approval and consent to participate
Not applicable.

Competing interests
The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential Conflict of interest.

Received: 8 April 2024 Accepted: 30 July 2024
Published online: 12 August 2024

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