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[redacted]

To Mr. Vice President
Christof Diefenbach

Hessian State Office
for Health and Care
PO Box 2913
65019 Wiesbaden

Hamburg, 29.04.2023

Application according to § 48 HVwVfG for withdrawal of the Manufacturer's Authorization DE_HE_01_MIA_2022_0087 of Biontech Manufacturing Marburg GmbH (ORG-100001653 / LOC-100002178) regarding the mRNA active pharmaceutical ingredient (API) BNT162b2 (mRNA-COVID-19 vaccine BioNTech)

Dear Mr. Diefenbach,

On April 10, 2023, an article by US researcher Kevin McKernan and his colleagues *was published which reported the massive DNA contamination of the mRNA-COVID-19 vaccines from BioNTech and Moderna, far in excess of the limit set by the European Medicines Agency (EMA). It is particularly shocking that even complete functional bacterial plasmids (ring-shaped DNA from genetically modified bacteria) were found in all vaccine samples.*

According to the current state of scientific knowledge, there is generally a well-founded suspicion that DNA contaminations of an injectable medication have harmful effects that go far beyond an acceptable level, even when the medication in question is used as intended. From a scientific point of view, this finding must therefore be described as highly questionable within the meaning of Section 5 of the German Medications Act. Details can be found in the attached document "Evaluation of the McKernan et al 2023 publication: Specification of DNA impurities in mRNA vaccines".

The responsibility of your office for correspondingly necessary inspections and consequences derives from the Act on the Establishment of the Hessian State Office for Health and Care of December 9, 2022 (LAGesPflErG HE) and the manufacturer's authorization DE_HE_01_MIA_ 2022_0087 of Biontech Manufacturing Marburg GmbH (ORG-100001653 / LOC-100002178) with regard to the production of BNT162b2 (mRNA-COVID-19 vaccine BioNTech) in Marburg (Hesse).

The above-mentioned Manufacturer's Authorization for Human Medicinal Products could only be granted if it was ensured that the requirements contained in Directive 2001/83/EC were met, just like the analogous regulations of the German Medications Act (AMG). Article 40 of Directive 2001/83/EC states that the manufacture of medications is subject to official authorization. Article 41 specifies the conditions for granting such a license. These include, in particular, that the applicant has suitable and sufficient control facilities.

In this respect, Article 20 obliges the competent authority - in this case, the Department II 23.2 of the Darmstadt Regional Council until December 31, 2022 and, since January 1, 2023, the Hessian State Office for Health and Care - to verify that the requirements for the granting of the Manufacturer's Authorization are met. The scope of these verifications is specified in Article 8 (3) h), where "biological examinations" are explicitly named and consequently the examination for biological impurities, which include DNA contaminations.

Directive 2001/83/EC is supplemented by Regulation (EU) No. 1252/2014, Article 12 (2) of which requires laboratory controls to be carried out to ensure compliance with the specifications laid down in accordance with Article 12 (1) for the quality and purity of the active substances manufactured, and also of the raw materials, starting materials and intermediates. Article 3 of Regulation (EU) No. 1252/2014 also requires the manufacturer to establish an effective system for quality management. This system, in turn, according to the regulation, must ensure in particular that the active ingredients meet the specifications laid down in accordance with Article 12(1) in terms of their quality and purity.

From my attached analysis based on the findings of McKernan and colleagues, you can now see that DNA contamination levels several orders of magnitude above the established threshold for DNA contamination have been detected in samples of vaccines containing the active ingredient BNT162b2. In addition, you can see from my analysis why, therefore, an exceedingly serious public health threat should be postulated for all vaccines containing the active ingredient BNT162b2.

Although it is not known in which production facility the samples examined by McKernan and colleagues were manufactured, the closer circumstances suggest that the DNA contaminations found are not the results of coincidences, but must be system-immanent deficiencies of the manufacturing process. The very fact that both the mRNA-COVID-19 vaccine from BioNTech and that from Moderna exhibit the problem of contamination with DNA in plasmid form suggests that mRNA agents for whose production linearized plasmid DNA is used as starting material generally cannot be produced with the required purity with regard to DNA contamination.

However, if such a general contamination problem exists, it must be assumed that also in Marburg for the production of the active ingredient BNT162b2 the granting of a Manufacturer's Authorization was unlawful in the sense of § 48 HVwVfG and must therefore be revoked.

In particular, it must also be determined whether the manufacturing authorization specified above was the result of fraudulent deception or a lack of due diligence on the part of the issuing authority staff. This, in turn, makes it imperative that all batches of BioNTech's mRNA-COVID-19 vaccine manufactured in Marburg be re-examined for contamination of any kind, and in particular with respect to DNA in general and plasmids in particular.

Since there is an imminent danger to public health in this case, immediate action by your authority is required. In this context, it is necessary to exclude the employees of your authority and of the Paul Ehrlich Institute, who were originally and in the broadest sense involved in the clarification of the existence of all prerequisites for the granting of the relevant manufacturer's authorization, from the now necessary investigations due to bias. Under this premise, in particular, the unannounced inspections prescribed under Section 64(3) sentence 4 on the basis of my indications of serious defects in the active substance BNT162b2 and the official examination of medication samples prescribed under Section 64(3) sentence 3 are to be carried out.

Of course, for the sake of completeness, the question of a possible systematic error in the studies by McKernan and colleagues must also be raised, even if the published data rule this out with a high degree of probability. However, I can already say that in the meantime other scientists (who do not wish to be named) have carried out less detailed, but significant investigations with mRNA vaccines marketed in Germany, which have also confirmed massive DNA contamination.

However, whatever remains to be determined, it is imminent danger and therefore acutely essential that the Manufacturer's Authorization DE_HE_01_MIA_2022_0087 relating to the pharmaceutical mRNA active pharmaceutical ingredient (API) BNT162b2 (mRNA-COVID-19 vaccine) of Biontech Manufacturing Marburg GmbH (ORG-100001653 / LOC-100002178) be withdrawn in order to prevent further serious harm to the public. I hereby request that this be done.

Finally, I would like to refer to the principle of the lawfulness of administration in **Article 20 of the Constitution**, which simply reads: "***The executive power and the administration of justice are bound by law and justice***".

It may be unusual for an application such as this to end with such a reference. However, the mere fact that it takes a scientist in the USA to uncover such a massive dubiousness of a medication marketed by the German state itself is capable of shaking confidence in what the fathers of the constitution called the "executive power" to its foundations.

I therefore appeal to you to confront this now directly and consistently - as the people of Germany are entitled to do. And by that I also mean your family, your friends, your neighbors.

With best regards

Attachment

[redacted]

Specification of DNA impurities in mRNA vaccines: Evaluation of the publication McKernan et al 2023

Publication details

Title:	***Sequencing of bivalent Moderna and Pfizer mRNA vaccines reveals nanogram to microgram amounts of expression vector dsDNA per dose***
Date:	**April 10, 2023**
Authors:	**McKernan, Kevin; Helbert, Yvonne; Kane, Liam T. und McLaughlin, Stephen** Medicinal Genomics, 100 Cummings Center, Suite 406-L, Beverly Mass, 01915, USA
Source:	**OSF Preprints, doi:10.31219/osf.io/b9t7m**

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Attachment

I. Abstract

The present evaluation concerns the experimental results of an analysis of BioNTech's mRNA-COVID-19 vaccine with respect to DNA contamination published by McKernan and colleagues in April 2023[1]. The comparable results on Moderna's mRNA-COVID-19 vaccine contained in the same publication are not included below for greater clarity.

The authors of the publication McKernan et al 2023[1] analyzed circulated batches of BioNTech's mRNA-COVID-19 vaccines with regard to DNA impurities found therein according to the authorities[2] and detected significant amounts of double-stranded DNA (dsDNA), partly in the form of complete bacterial plasmids. Plasmids are small ring-shaped carriers of genetic information that are independent of the much larger, also ring-shaped "bacterial chromosome" and carry all the information necessary for the expression of the genes on them, i.e. the formation of gene products (proteins) of the genes located on the plasmid. The origin of these DNA contaminations can be traced back to the production process[3].

According to sequencing, the genes found on the detected plasmids are in particular the gene for the spike protein of the SARS-CoV2 virus, but also a gene for antibiotic resistance, the functionality of which was confirmed experimentally. The fact that plasmids were found not only in BioNTech's mRNA-COVID-19 vaccine, but also in Moderna's, suggests that insufficient removal of DNA impurities in these vaccines is a fundamental problem in the manufacture of this class of products.

McKernan and colleagues also determined the quantitative composition of the nucleic acids (DNA and RNA) contained in the vaccine. They found that the DNA contaminants in the BioNTech vaccine exceeded the European Medicines Agency (EMA) limit by a multiple of 84 to 1445 times, depending on the sample and methodology.

In light of this, the risk profile of the massive DNA contamination found by McKernan and colleagues in BioNTech's mRNA-COVID-19 vaccine is as follows:

1. The risk of non-reversible integration of foreign DNA from an mRNA vaccine into the genome of the vaccinated, which is associated with the risk of alteration of human genes (insertional mutagenesis). In particular, the risk of carcinogenesis should be mentioned.
2. The risk of a long - possibly even lifelong - production of the spike protein in the body of the vaccinated.
3. The risk of antibiotic resistance in the body of the vaccinated.

The risks of DNA contamination found by McKernan and colleagues in BioNTech's mRNA-COVID-19 vaccine are of high concern by scientific standards. It is therefore necessary to demand that the production process of the vaccine in question be completely reconsidered with the aim of completely eliminating DNA contamination of the final product. For plasmids, the zero limit must be established. As long as this is not achieved, it must also be assumed, beyond the scientific assessment, that there is a concern in the legal sense according to Section 5 of the German Medications Act. This means that batches of BioNTech's mRNA-COVID-19 vaccine for which there is no explicit proof of the absence of DNA contamination may neither be used nor put on the market.

II. Introduction

Immediately after approval of BioNTech's mRNA-COVID-19 vaccine, the European Medicines Agency (EMA) published a review report that acknowledged the presence of DNA impurities in the marketed product[(2)]. Linearized DNA was mentioned, but not circular plasmids. Furthermore, no further specification was given, neither by type nor by amount of these DNA contaminations. However, it was clarified that these DNA contaminations originate from the production process. Thus, the production process plays a key role in the evaluation of the facts on which this report is based. In this sense, the following explanations follow the source (3) for the BioNTech product.

In order to produce mRNA as an active ingredient in medications, its genetic blueprint is used as a DNA template from which the mRNA is then repeatedly "transcribed" enzymatically and thus amplified. According to the published approval documents (EMA Assessment Report)[(2)], the approval studies of BioNTech's COVID-19 mRNA vaccine used a vaccine preparation whose DNA matrices were produced by machine in the laboratory (PCR). This method represents the current state of science. This also applies to the subsequent purification of the mRNA of the study vaccine using the so-called magnetic bead technology and thus with a high standard. This manufacturing route is designated "Process 1" by the EMA. However, both technologies mentioned, the machine generation of DNA templates by PCR and the purification using magnetic beads, are cost-intensive.

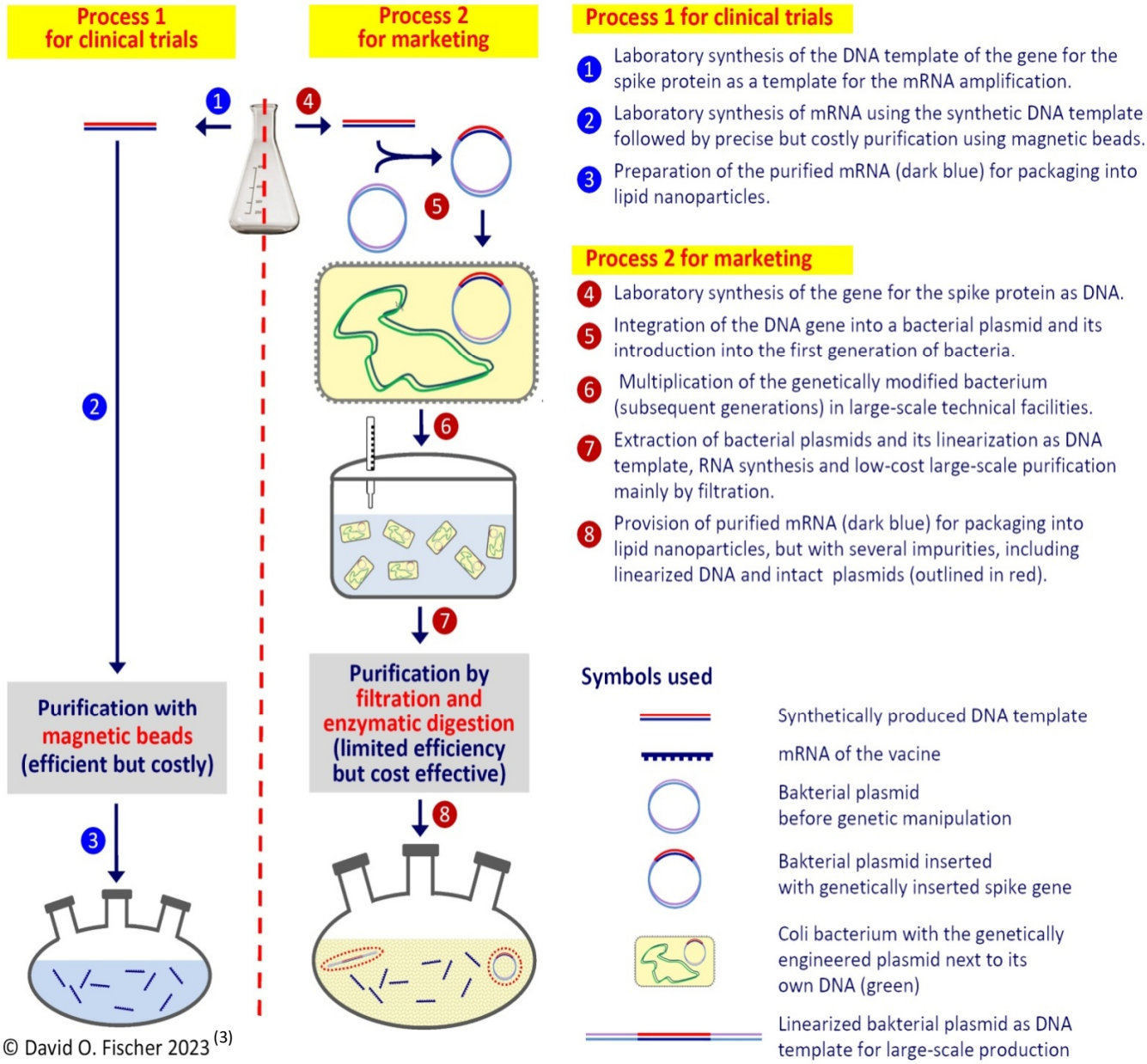
In order to save costs and thus increase the profit margin, the manufacturing method for the commercially marketed product was fundamentally simplified. However, in exchange for this cost advantage, an increased risk of contamination of concern was accepted, as this is the disadvantage of the manufacturing route referred to by the EMA as "Process 2". However, this brings with it the risk of considerable contamination of various kinds, which is much lower in the case of the "Process 1" manufacturing method used for the trial medication.

This becomes clear when "Process 1" and "Process 2" are compared (Figure 1). Magnetic beads are magnetized particles which, depending on the design of the surface, bind the substance to be extracted from a sample, in this case specifically the mRNA, and thus virtually fish it out. These magnetic beads are added to the sample, where the mRNA attaches to the surface of the beads. Using a magnetic field, the beads with the attached mRNA are then held to the wall of the sample vessel while the liquid with the impurities remaining in it is aspirated. A contamination-free aqueous solution is then added to the sample and the magnetic field is turned off. This allows the beads to disperse into the liquid and release the mRNA into it. The result is a largely contamination-free mRNA preparation.

However, the far more cost-effective production according to "Process 2" uses much less expensive filtration processes. These, in contrast, can only separate larger molecules from smaller ones, and with limited efficiency. A "specific fishing out" as allowed by the use of magnetic beads is not possible in this way. The risk of contamination that arises from the use of filtration methods instead of magnetic bead technology is further increased in "Process 2" by the use of synthetic DNA matrices derived from plasmids of genetically manipulated coli bacteria instead of the synthetic DNA matrices produced in the PCR process in "Process 1". Plasmids are ring-shaped strands of DNA that carry the gene for the spike protein, but also genes that are useful for production. These often include antibiotic resistance genes, so that by adding the respective antibiotic to the culture solution, undesirable foreign bacteria are killed, but the desirable bacteria containing the plasmid with the antibiotic resistance gene survive.

The parent plasmid, genetically engineered according to these specifications, is introduced into a first-generation bacterium and then propagated in a culture solution together with the coli bacteria modified with it. The genetically modified bacteria grown in this way are then dissolved and the plasmids released with them are concentrated by filtration. The ring form of the plasmids is then broken up to form a linear strand. This then provides the DNA template for large-scale mRNA production. This is the theory.

Figure 1: Production of BioNTech's mRNA vaccine



The analyses of McKernan and colleagues, on the other hand, have now shown that in practice it is apparently not possible to keep the batches of mRNA-COVID-19 vaccines prepared according to the process referred to by the EMA as "Process 2" free from double-stranded DNA and, in particular, from whole plasmids.

The aim of the present critical evaluation is now to reach a scientifically based assessment of the risk profile of DNA contaminants found in mRNA-COVID-19 vaccines, based on the methodology and results of the publication of McKernan et al 2023[(1)].

III. Methodological approach of McKernan and colleagues and evaluation of the respective results

Isolation of possible DNA contamination of the mRNA-COVID-19 vaccines

Depending on the forms and quantities of DNA present in the samples to be tested, different risk profiles for humans are derived. In order to be able to characterize DNA contaminations of the samples, it is first necessary to remove the RNA so that it does not falsify the DNA determination. This was done using the enzyme RNase A (Monarch RNase A, New England Biolabs), which is known to degrade even the mRNA modified with building blocks not found in nature (mod-mRNA) of BioNTech's COVID-19 mRNA vaccine. The dsDNA remaining after this step can occur linearly (DNA strand) or as a circular plasmid. To investigate the type and amount of DNA remaining, it was first separated from the remaining components (lipids, possibly proteins) using a commercially available kit (SenSATIVAx, Medicinal Genomics) and purified. The purified DNA was then subjected to several test procedures for further characterization.

Test of the isolated DNA for plasmid properties

It was investigated whether the purified DNA can be taken up by bacteria and their genes expressed, i.e. whether the proteins encoded on the DNA can be produced. For this purpose, purified DNA was incorporated into E. coli bacteria (modified strain B21, #C2523I, New England Biolabs) using the heat shock method. The heat shock, which lasted for 20 seconds, briefly caused perforations in the bacterial envelope, allowing the foreign DNA to enter the interior of the bacteria. After a brief recovery for the bacteria, they were placed on culture plates containing nutrient medium. The culture medium contained the antibiotic kanamycin, so that only bacteria that had taken up a kanamycin gene in the form of foreign DNA could grow into colonies on it. Since such colonies appeared, it must be considered proven that the DNA isolated from the vaccines carries the gene for kanamycin resistance.

Significance of the results:

The DNA that had been taken up by the bacteria and led to resistance to Kanamycin must have been present at least in part as a circular plasmid, since as a rule only this, and not linear DNA, is expressed in the given test system. DNA of a bacterial chromosome (genomic DNA) would be too large to enter bacteria by heat shock method.

Confirmation of the presence of plasmids

Bacteria from the colonies obtained according to the method described in the previous section were taken up in water and subjected to lysis. During this lysis, the bacterial envelope is destroyed to gain access to the DNA. To ensure that the E.coli bacteria used and not foreign bacteria derived from contamination were extracted, the PathoSEEK E.coli detection assay kit (#420102, Medicinal Genomics) was used as a positive control. Subsequently, DNA isolated from the bacteria was analyzed for genomic DNA and the presence of plasmid DNA using an Agilent Tape Station. The Agilent Tape Station is an automated standard method to determine the quality, size and integrity of DNA and RNA. This test method confirmed that the DNA extracted from the vaccine samples and ingested by the bacteria is indeed plasmid DNA.

Significance of the results:

Plasmids that carry an antibiotic resistance gene, such as those detected here, are known in genetic engineering as cloning vectors. These cloning vectors are used to enrich specific genes in bacteria, such as genes for the spike protein of SARS-CoV-2. The more such plasmids are contained in a bacterium, the more effectively it contributes to obtaining DNA templates for mRNA production.

Plasmid sequencing

The isolated plasmids were now sequenced using the "Whole Genome Shotgun Method". In this method, the DNA of the plasmids is broken down into small fragments, which in turn are introduced into cloning vectors. This results in numerous vectors, each of which contains only one DNA fragment and is accessible to automated sequencing. The result of this sequencing is short nucleotide sequences corresponding to the fragments, but overlapping at the ends. With the help of special software, it is possible to assemble sequences with matching overlap like puzzle pieces into a total DNA sequence (Figure 2).

Significance of the results:

This procedure made it possible to determine the entire sequence of the plasmid vector derived from BioNTech's COVID-19 mRNA vaccine. The gene for kanamycin resistance can also be read off in this sequence. This gene is activated by a special sequence that is frequently used in genetic engineering, the so-called SV40 promoter. The enzymes required for transcribing the mRNA from the DNA are attached to the promoter, whereby the specific characteristics of the promoter define its regulation, which is fundamentally different from the regulation of other promoters. This is also the case for the promoter T7, which controls the activation of the gene for the spike protein of the SARS-CoV2 virus, which was also found as expected.

The mere presence of plasmids in the batches released for vaccination is evidence of inadequate purification of mRNA prior to its packaging in lipid nanoparticles (LNPs). Since even a small contamination of vaccines with DNA already poses considerable risks to the vaccinated, this circumstance must be classified as extremely alarming.

Ultimately, during the production of lipid nanoparticles (LNPs) for the ready-to-apply mRNA vaccine, it is not possible to control whether mRNA or DNA or both are packaged in different proportions in the lipid envelope of the LNPs. Human cells also do not differentiate in this regard. DNA-containing LNPs are taken up by the cells of the vaccinated in the same way as LNPs containing only mRNA. Thus, the content of the LNPs does not determine the specificity and amount of uptake by the cells of the human recipient. Thus, after vaccination, the DNA impurities also enter the cells of the vaccinated.

The genes found on the plasmids encode proteins that can also exert unwanted functions in human cells. For example, McKernan and colleagues found an SV40 promoter on the plasmid found in BioNTech's mRNA-COVID-19 vaccine, which activates the gene for kanamycin resistance. The SV40 promoter originated in Semian virus 40 and is constitutively active in eukaryotic cells, which include all animal and human cells. This means that the gene located downstream of an SV40 promoter - in this case, the kanamycin resistance gene - establishes resistance to the antibiotic kanamycin in the human cell, but also cross-resistance to other antibiotics of the same group of aminoglycosides, such as gentamycin, neomycin, streptomycin and dibekacin.

Furthermore, a so-called f1 ori sequence is found on the plasmid. This sequence is required to replicate the plasmid DNA in the bacterial cell. However, the f1 ori sequence can also act as a starting point for replication of the plasmid DNA in human cells if a gene for the protein called "large T antigen" or a homologue derived from a polyomavirus is also present in the affected cell. The polyomavirus contamination in humans is up to 90 %. This in turn means that a large proportion of people have persistent polyomaviruses, which in turn have the gene for a "large T antigen" protein (or a homologue).

Hence, if a plasmid from the vaccine with the vaccine nanoparticles is introduced into a human cell, which in turn is infected with a persistent polyomavirus, this will lead to a proliferation of the plasmid in the affected human cell and thus to an uncontrolled and probably also long-lasting expression of the spike gene. Thus, contamination of plasmid DNA from the mRNA manufacturing process for vaccine production may be a possible explanation for the long-lasting expression of the vaccine spike protein[(5)].

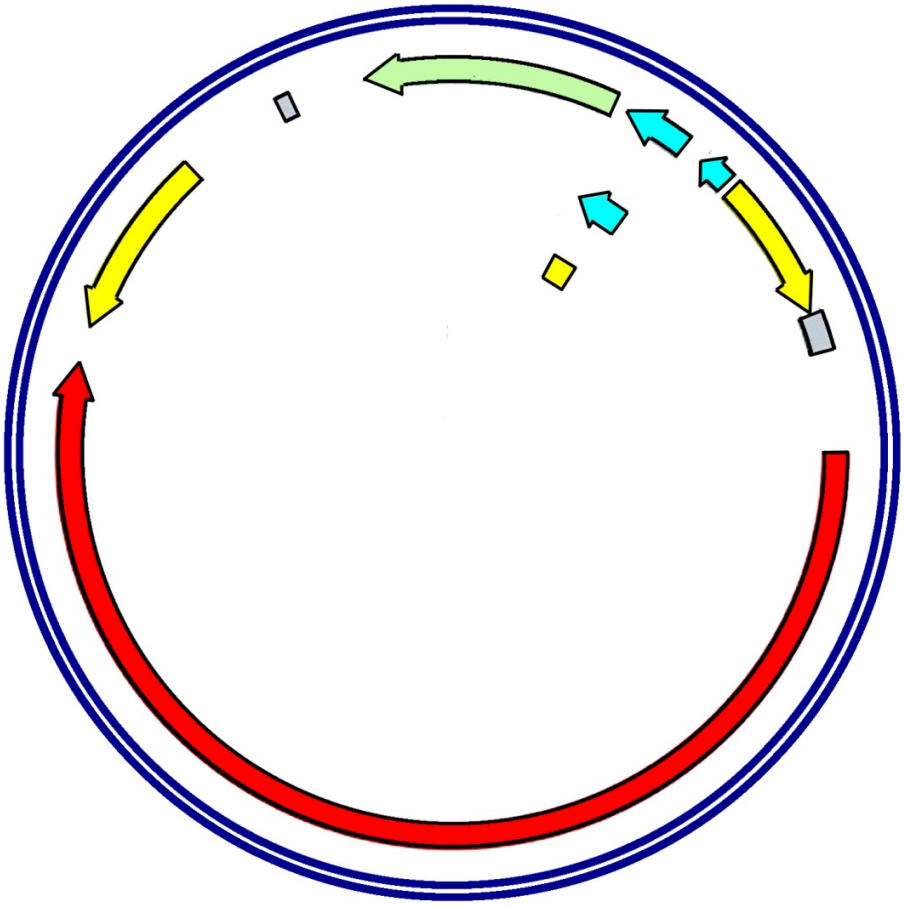
Determination of the plasmid identity

In principle, there is a possibility that more than one plasmid variant is hidden in a sample. Quantitative PCR analysis (qPCR) makes it possible to investigate this. For this purpose, McKernan and colleagues selected two defined regions of the sequenced plasmid - once the region of the gene for the spike protein and additionally the region of the gene for kanamycin resistance. In the qPCR, both areas were quantified separately In the result, the detected ratio is 1:1, so that it can be assumed with a certain probability that only one plasmid variant was present in the samples.

Significance of the results:

The values determined by qPCR indicate that the samples tested are contaminated with a plasmid vector in unexpectedly high quantities.

Figure 2: Sequence of a plasmid found by McKernan and colleagues in BioNTech's mRNA-COVID-19 vaccine (modified from Figure 2[(1)]).



Gene for the spike protein of the virus SARS-CoV2

Gene for kanmycin resistance

Regulatory sequences ensuring proper functioning of the plasmid

Quantitative determination of the nucleic acid content

For BioNTech's mRNA-COVID-19 vaccine, McKernan and colleagues determined DNA and RNA content using two standard methods, Agilent Tape Station™ electrophoresis and Qubit™ 3 fluorometry:

BioNTech **Agilent** Figure 6 [1]	DNA ng/µl	DNA ng/Dose	RNA ng/µl	RNA ng/Dosis	Nucleic acids ng/Dose	Nucleic acids % DNA	DNA /RNA	ngDNA /1mg RNA	Multiple of the limit value of 330 ng DNA per 1 mg RNA*
Sample 1	11,30	3390	23,70	7110	10500	32,29	0,48	476793	1445
Sample 2	8,19	2457	28,30	8490	10947	22,44	0,29	289399	877

BioNTech **Qubit** Table 1 [1]	DNA ng/µl	DNA ng/Dose	RNA ng/µl	RNA ng/Dosis	Nucleic acids ng/Dose	Nucleic acids % DNA	DNA /RNA	ngDNA /1mg RNA	Multiple of the limit value of 330 ng DNA per 1 mg RNA*
Sample 3	2,81	843	30,00	9000	9843	8,56	0,09	93667	284
Sample 4	1,47	441	52,80	15840	16281	2,71	0,03	27841	84

* Limit value as set by the EU Medicines Agency EMA:	330	ng DNA per mg RNA [4]
	10	ng DNA per 30 µg RNA (1 Dose) [4]

Hence, depending on the sample and method, 84- to 1445-fold of the permissible limit for DNA contamination in BioNTech's mRNA-COVID-19 vaccine for the EU was detected.

Significance of the results:

The authors McKernan et al 2023 themselves admit that their results are of a preliminary nature. Thus, only samples that had already expired were used. It is therefore to be expected that more valid results will be presented by McKernan in the foreseeable future.

Despite these limitations, the analyses performed so far by McKernan and colleagues are highly alarming. The DNA contamination found in the BioNTech vaccine with basically functional DNA exceeds the limit specified by the EMA many times over. However, this DNA content is not disclosed either in its directions for use or in the expert information, since ingredients of a medication declared as impurities do not have to be disclosed. In this respect, however, it must be questioned whether this is actually a case of contamination with DNA, or whether the DNA does not also contribute to the pharmacological effect of this vaccine, since it is a carrier of the activatable gene of the spike protein. Thus, this DNA is in principle suitable to force cells of the vaccinated person to produce the spike protein - in addition to the spike protein that is formed on the basis of the introduced mRNA. According to the available findings, this can be assumed to be the case at least for the vaccine product that is manufactured using the process referred to by the EMA as "Process 2", i.e. the commercially marketed vaccine. However, this in turn means that the DNA components of the vaccine, which are currently referred to as impurities, would have to be identified as an additional active ingredient.

IV. Evaluation of the DNA contamination risk profile of BioNTech's mRNA-COVID-19 vaccine

Contamination with plasmids - a permanent quality problem of "Process 2"

According to the EMA's Assessment Report[(2)], the plasmids derived from coli bacteria for the production of BioNTech's mRNA-COVID-19 vaccine according to "Process 2" are linearized prior to mRNA production. This is a step necessary for transcription and thus mRNA production. However, the presence of whole plasmids in the final product, as demonstrated by McKernan and colleagues, shows that linearization is regularly highly incomplete. It is therefore unlikely that this was not noticed during the quality controls in the production process.

If the incompleteness of the linearization of the plasmids is accepted by BioNTech, but also by the monitoring authorities, this speaks for the fact that in this respect there is a fundamental problem in the production, which obviously could not be solved. The fundamental nature of the contamination of the mRNA vaccines with plasmids is clear from the fact that this problem applies not only to the mRNA vaccines marketed by BioNTech, but also to those marketed by Moderna[(1)].

Apparently, no consequences were drawn from this, however, and instead capitulated in the face of this problem. Thus, it must be assumed that the production of mRNA vaccines according to "Process 2" for large-scale production can only be carried out inadequately with a high degree of dangerous contamination already with regard to the provision of the DNA matrices. This in turn calls into question whether the specifications of "Good Manufacturing Practice" (GMP) with regard to "Process 2" are adhered to at all.

The next step after completion of the transcription process, i.e. the generation of mRNA, is the degradation of the now no longer necessary linearized DNA plasmids by enzymes (DNases) into nucleotides in order to eliminate the contamination of the final product with DNA already at this point of the manufacturing process "Process 2". The degradation products, i.e. the now separated nucleotides of the DNA, are to be separated during the subsequent filtration. However, the very high contamination of released vaccine batches with DNA shows that the DNA digestion step in "Process 2" is also highly inadequate. Thus, after the insufficient linearization of plasmids with the insufficient digestion of DNA by DNases, there is a second serious production problem, which questions the safety of the final product in an alarming way.

Already in its first published Assessment Report[(2)] the EMA criticized the insufficient quality controls of the enzymatic digestion of DNA contaminations, but did not question the possible consequences for product safety and apparently did not take remedial action. The latter could also be an indication that "Process 2" is unsuitable for mass production and that the EMA silently accepted the resulting DNA contaminations in order to enable the availability of the final product - even if this questionably compromises product safety. However, it may also be the case that the production of mRNA vaccines based on bacterial plasmids according to "Process 2" is generally unsuitable for upscaling, i.e. large-scale production.

The risk of insertional mutagenesis

It is known that the lipid nanoparticles of the mRNA vaccines and thus also the DNA contaminations contained in them can in principle penetrate into all human cells, so that an integration of the plasmid DNA found by McKernan and colleagues in the mRNA-COVID-19 vaccine into the human DNA, which then takes place there, cannot be excluded from the outset. Furthermore, it has been known for decades that the introduction of a foreign DNA into human cells offers the organism the basic possibility to stably and irreversibly insert this foreign DNA into the human genome Since this ultimately always represents a mutation of the human genome, this process of insertion of foreign DNA is referred to as insertional mutagenesis[(6)].

The process of insertional mutagenesis and the associated mutations are the subject of intensive research. The genotoxic effects of foreign DNA resulting from insertional mutagenesis are basically divided into three groups:

a) Gene inactivation: The insertion of DNA into the host genome can occur within a gene, thereby preventing the gene from functioning. This can lead to the loss of essential proteins in the cell and potentially to the development of a wide variety of diseases, including cancer[(6)].

b) Gene activation: Certain regulatory and other genomic sequences can be activated. This can increase the synthesis of certain proteins in the cell, which also poses a cancer risk[(6)]. Since the new cancer cells generated in this way can mature into clinically manifest tumors, this type of integration has now become a common technique in tumor biology[(7)].

c) Gene regulation: Transcriptional and epigenetic regulatory mechanisms may be impaired, resulting in dysregulation of protein expression with unpredictable and undesirable results[(6)].

The occurrence of malignancies due to DNA integration and consequent oncogene activation was already demonstrated in 2000 in a clinical trial using a retroviral vector to treat children with SCID-X1 (severe combined immunodeficiency)[(8)(9)]. At that time, it was found that three years after treatment, 2 out of 10 children treated in this manner developed leukemia. The reason identified was the integration of DNA in the vicinity of a cancer gene. Accordingly, the authors of this study already cautioned at that time that an observation period of 6 months after treatment with DNA agents is not sufficient to detect long-term side effects of this kind.

This makes clear how essential thorough and long-term studies on possible genotoxic effects are also for the foreign DNA that may be introduced into the human organism with the mRNA-COVID-19 vaccine from BioNTech. However, these have been lacking to date, with the express permission of the EMA and the EU Commission[(2)]. However, the massive DNA contamination now found by McKernan and colleagues means that this issue is now becoming acute at the level of local regulatory authorities, who are responsible for overseeing the production facilities from which the DNA contamination originates. They must now immediately review whether the production permit required for each site can be sustained.

The risk of prolonged expression of the spike protein

The T7 promoter, located upstream of the gene encoding the spike protein on the plasmid found by McKernan and colleagues in BioNTech's mRNA-COVID-19 vaccine, plays an essential role in the activation of this gene. However, because the T7 promoter has not been finally characterized scientifically, it cannot be ruled out that, depending on specific circumstances, an undetermined amount of the spike protein may be read from the plasmid DNA and synthesized by the human cell. Possibly, this could be an explanation for the long-lasting detectability of spike protein in the body, which has already been observed several times in vaccinated individuals.

The risk of integration of intramuscularly administered plasmid DNA into genomic DNA was first demonstrated in a mouse model in 2004[(10)]. Since then, it has been shown that integration of plasmid DNA into the host genome becomes possible when the host cell enters cell division. Cells that divide in this way in humans are, in particular, the stem cells and progenitor cells of all organs. In particular, skin cells, cells of the gastrointestinal tract, blood cells and cells of the bone marrow undergo constant and rapid cell divisions. Each cell division involves the dissolution of the cell nucleus and thus the exposure of the chromosomal (genomic) DNA of the cell, so that plasmid DNA can come into close contact with the chromosomal DNA of the human and be integrated into the genome by appropriate mechanisms of the human cell.

Overall, the mechanism of insertion of foreign DNA into mammalian cells is not yet understood to the required extent at the molecular level. Accordingly, there is still a great need for research in this regard. In particular, the question of the persistence of plasmids once inserted into a division-competent human cell is also significant in this context. The longer they persist, the higher the risk of insertional mutagenesis, i.e. the risk that the plasmids carrying the gene for the spike protein will be integrated into the genomic DNA of the inoculated and from there an uncontrolled production of spike protein will take place.

Although the risk of integration of plasmid DNA into the genomic DNA of humans or animals has been known for decades, there are still insufficient data on the frequency of this occurrence or the essential factors in this respect. As long as this remains the case, an estimation of this risk can be made, at least in the alternative, on the basis of data collected on the frequency of integration of adenovirus DNA. In this sense, for example, a study conducted in mice in 2022 came up with an integration frequency of up to 0.005 per cell (liver cells into which the adenovirus had entered). This means that in one out of 200 cells infected with the adenovirus, integration of the viral DNA into the genomic DNA and thus insertional mutagenesis occurred[(11)].

This value is more than alarming if it must be assumed to be conceivable in the absence of more specific data for a marketed medication such as BioNTech's mRNA-COVID-19 vaccine. But that is precisely the case in light of the data presented by McKernan and colleagues on the contamination of this vaccine with DNA. For every medication on the market, however, proof of safety must have been provided, otherwise the marketing, but also the treatment with it, is punishable under the German Medicines Act (§ 5 AMG in conjunction with § 95 AMG).

In this context, it is essential that current FDA recommendations state that a long-term follow-up study (LTFU) of up to 15 years is required for medications that can integrate into the genome[12]. This must include investigation of new-onset malignancies, hematologic disorders, onset or exacerbation of neurologic disorders, autoimmune disease, or potentially product-related infections.

So far, these state-of-the-science guidelines have not been implemented with regard to mRNA vaccines - apparently in ignorance of the massive contamination of these medications with DNA and, in particular, in the form of plasmids. Now, however, after McKernan and colleagues have demonstrated and made public the massive DNA contamination of these vaccines, a rethink must urgently take place in this regard. In this sense, the strict standards that are applied to other medications containing nucleic acids potentially integrating into the human genome must also be applied to mRNA vaccines without delay.

The risk of antibiotic resistance in the body of the vaccinated

McKernan and colleagues identified not only a gene for the spike protein but also a gene for kanamycin resistance[1] on the plasmid found in BioNTech's mRNA COVID-19 vaccine. However, this gene can also establish resistance to the antibiotic kanamycin in human cells, and even more so cross-resistance to other antibiotics of the aminoglycoside group, such as gentamycin, neomycin, streptomycin and dibekacin.

Such cellular multiresistance to aminoglycosides could spread to harmful bacteria that have invaded the body by horizontal gene exchange processes, which in turn could make it considerably more difficult to combat them. Such endogenous resistance of humans to antibiotics therefore also poses a serious risk, which according to current knowledge may have unforeseeable consequences.

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Attachment

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