OVERRIDING PUBLIC INTEREST REPORT

CONCERNING RESIDUAL DNA

IN COMIRNATY AND SPIKEVAX

Submitted within the framework of the

Transparency4Safety Initiative (T4SI)

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1 Introduction

This report is submitted within the framework of the Transparency4Safety Initiative (T4SI), which seeks access to the regulatory evidence underlying the authorisation and continued marketing of modRNA medicinal products in order to enable independent scientific and regulatory verification.

It substantiates an overriding public interest in disclosure under Article 4(2) of Regulation (EC) No 1049/2001 in relation to regulatory documents concerning residual DNA in the centrally authorised modRNA vaccines Comirnaty and Spikevax, as well as all other modRNA medicinal products.

Residual DNA is a process-related impurity arising from the DNA template used for the manufacture of the modRNA drug substance. Its regulatory control concerns not only the amount of residual DNA, but also its fragment size, sequence, biological activity, removal during manufacture, analytical detectability and relevance in the final LNP-formulated medicinal product. These parameters directly concern the quality and safety assessment on which the marketing authorisations depend.
Since the initial authorisations, independent studies, subsequent WHO and EMA guidance, new pharmacopoeial standards and further regulatory disclosures have raised material questions as to whether the originally applied residual DNA control strategy, including reliance on qPCR and the historical 10 ng/dose threshold, is scientifically adequate and product-specific for modRNA-LNP medicinal products.

These unresolved questions must also be assessed in light of the precautionary principle, as reflected in Article 191(2) TFEU and recognised as a general principle of Union law applicable to the protection of human health. In particular, the scientific and regulatory novelty of modRNA-LNP medicinal products requires potential risks to be investigated even where their existence or extent cannot yet be established with certainty. Where access to the underlying regulatory evidence is necessary to examine such uncertainty, the precautionary principle reinforces the overriding public interest in disclosure and requires the protection of human health to take precedence over purely economic interests, as recognised by the Court of Justice in *National Farmers' Union* (C-157/96), *United Kingdom v Commission* (C-180/96), and *Monsanto Agricoltura Italia* (C-236/01), as well as in the General Court's judgment in *Artegodan* (T-74/00) and the Court of Justice in *Bayer CropScience* (C-442/09).

The purpose of this report, as part of the T4SI, is to identify the scientific and regulatory issues requiring independent verification and the underlying regulatory documents necessary for that verification. It addresses, in particular, the analytical methods and their validation, DNA-template and plasmid characterisation, DNase digestion and purification, residual DNA fragment size and sequence, batch-level results, drug-substance (DS)/drug-product (DP) bridging, LNP-related matrix effects and the regulatory reasoning supporting the approved control strategy.

The requested disclosure is necessary to determine independently whether the authorised products comply with applicable Union quality and safety requirements and whether the conditions for maintaining their marketing authorisations remain fulfilled.

Where access to the underlying evidence is indispensable for such verification, the protection of commercial interests under Article 4(2) of Regulation No 1049/2001 must yield to the overriding public interest in protecting human health, ensuring scientific verifiability and securing regulatory accountability.

2 Scientific Findings on Excess Residual DNA

In February 2022, *Aldén et al.*[1] reported that the modRNA contained in the COVID-19 vaccine from Pfizer/BioNTech (Comirnaty, BNT162b2) was taken up by the human liver cell line Huh7 within only six hours of exposure. It was demonstrated that the uptake triggered the expression of both LINE-1 (Long Interspersed Nuclear Element-1) and ORFp1 (Open Reading Frame RNA-binding protein-1), an enzyme capable of reverse transcribing modRNA into DNA. PCR analysis confirmed that modRNA from the Comirnaty vaccine may have been reverse transcribed into DNA within the cells of the human liver cell line, indicating the potential for genomic integration. A potential limitation that should be mentioned is that this study was performed prior to the detection of residual DNA in COVID-19 vaccine vials of both Comirnaty and Spikevax (Moderna, modRNA -1273) by McKernan (see next paragraph). Therefore, results reported by *Aldén et al.* (2022) may also be due to residual DNA, which has not been verified by the authors. However, the findings by *Aldén et al.* (2022) challenge prior regulatory assumptions that synthetic modRNA – and even residual DNA contained in the RNA-based genetic vaccines Comirnaty and Spikevax - is transient and incapable of interfering with or altering human DNA.

In April 2023, McKernan *et al.*[2] detected residual DNA from bacterial plasmids in both BioNTech/Pfizer and Moderna COVID-19 vaccines at levels far exceeding the regulatory safety threshold of 10 ng/dose[3]. In addition, authors demonstrated the presence of SV40 promoter/enhancer and nuclear localisation sequences in Comirnaty, but not in Spikevax, applying multiple detection methods:

- Electrophoresis (Agilent Tape Station): 2,250–3,390 ng/dose
- Fluorometry (Qubit): 312–843 ng/dose
- qPCR (targeting spike and plasmid origin of replication (Ori)): 12 ng/dose

The reported levels of residual DNA (ranging from 312–3,390 ng/dose, depending on the method used) exceeded the FDA´s/WHO's safety limits of 10 ng/dose by up to 339 times and the EMA's limit of 330 ng/mg RNA by up to 103 times for BioNTech/Pfizer and 31 times for Moderna.[4] It must be emphasized that the approved regulatory guidelines apply for naked DNA fragments ≤200 bp, but not for DNA fragments protected inside lipid nanoparticles (LNPs). To date, there is no known threshold for LNP encapsulated DNA. This work by *McKernan* and co-workers has been confirmed by multiple studies worldwide.

In October 2023, *Speicher et al.*[5] examined 27 COVID-19 vaccine vials from Ontario (Canada). Using qPCR, authors observed levels of residual DNA up to 4.27 ng/dose for Comirnaty and 0.78 ng/dose for Spikevax. Applying Qubit® fluorometry for total DNA, authors measured 1,896–3,720 ng/dose for Comirnaty and 3,270–5,100 ng/dose for Spikevax. This work has since been expanded to 32 vials drawn from 16 lots ([Comirnaty (10 vials; 6 lots) and (22 vials, 10 lots)] with DNA levels measured by qPCR of 0.22–7.28 ng/dose for Comirnaty, and 0.01–0.78 ng/dose for Spikevax. The SV40 promoter/enhancer-Ori was 0.25–23.72 ng/dose and was only detected in Comirnaty. Total DNA as measured by Qubit® fluorometry was 371–1,548 ng/dose and 1,130–6,280 ng/dose for Comirnaty and Spikevax, respectively. This study highlights the huge discrepancy of residual DNA loads

[1] *Aldén et al.* 2022 https://doi.org/10.3390/cimb44030073

[2] *McKernan et al.* 2023 https://osf.io/preprints/osf/b9t7m_v1

[3] US, Food and Drug Administration 2020. Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs) https://www.fda.gov/media/113760/download; *Shin et al.* 2005. WHO informal consultation on the application of molecular methods to assure the quality, safety and efficacy of vaccines, Geneva, Switzerland, 7-8 April 2005 https:// doi.org/10.1016/j.biologicals.2005.12.005

[4] McKernan Substack 2023 https://anandamide.substack.com/p/sequencing-of-bivalent-moderna-and

[5] *Speicher et al. 2023* https://osf.io/preprints/osf/mjc97_v1

depending on the applied method and, therefore, the need for the establishment of standardised methods.

When analysed by qPCR, there was a distinct difference in load depending on both primers and amplicon applied. In the Comirnaty vials analysed by *Speicher et al.,* Ori, spike, and SV40 promoter/enhancer were 0.28–7.28 ng/dose, 0.22–2.43 ng/dose, and 0.25–23.72 ng/dose, respectively. In the Spikevax vials, the difference between Ori and spike was 0.01–0.34 ng/dose, and 0.25 –0.78 ng/dose, respectively. The huge difference between the various Spikevax vials may at least in part be due to the nucleoside-modified modRNA (modRNA), which is bound to the spike sequence on the plasmid. These DNA:RNA hybrid sequences are well known to inhibit the enzyme DNase I from processing and removing this DNA.[6] Therefore, it is essential that multiple PCR targets are used.

For the quantification of total DNA, *Speicher et al.* performed Qubit® fluorometry directly on the drug product, after a heating step to disrupt the LNPs, followed by treatment with the enzyme RNase A to degrade RNA and assess potential crosstalk between the fluorometric DNA reporting dye AccuGreen® and modRNA. Heat denaturation would release both residual DNA and modRNA from the LNPs, and therefore increases the amount of measurable DNA. Notably, after RNase A treatment, a large amount of total residual DNA was detected in vaccine vials from both manufacturers. This suggests that a large fraction of the DNA fragments is below the qPCR amplicon size range (i.e., is degraded into short fragments), and that Moderna used a substantially higher amount of plasmid DNA for modRNA transcription.

Speicher et al. performed a size distribution analysis and found mean and maximum DNA fragment lengths of 214 bp (base pairs) and 3.5 kb (kilo base pairs), respectively. Thus, the size of the residual DNA fragments present in the COVID-19 vaccines are likely to be much larger than the regulatory limit of 200 bp.

In addition, *Speicher et al.* compared the DNA load with spontaneously reported suspected adverse events listed in the VAERS database and found that the early manufactured lots of BioNTech/Pfizer were linked to a significant increase in adverse events. This correlation is potentially due to the monovalent, purple-capped vials having a PBS buffer formulation that resulted in visible particles in the final drug product, which may be associated with toxicities including micro emboli and inflammatory reactions.[7] On October 29, 2021, the US FDA authorised a change of formulation from PBS to Tris/sucrose buffer.

In February 2024, *Kaemmerer* conducted an experiment in which OvCar3 cells were transfected overnight with Comirnaty and spike expression was documented by immunohistochemistry. These cells were sent to *McKernan,* who quantified the SV40 promoter/enhancer, origin of replication (Ori), and spike DNA in the OvCar3 cells after two serial passages, showing that the DNA was maintained through cell division. Sequencing of the cellular genomic DNA found putative integration events of DNA encoding the entire vaccine-derived spike ORF into chromosomes 9 and 12 of OvCar3 cells, with the latter integrated into the cancer suppressor gene FAIM2 (Fas Apoptotic Inhibitory Molecule-2).[8] This study showed that integration of DNA fragments from the Comirnaty vaccine into the human genome is possible, and that it is important to investigate whether integration can occur in primary cells in vivo in individuals from the vaccinated population.

[6] *Sutton et al.* 1997 https://doi.org/10.1042/bj3210481

[7] U.S. Department of Health and Human Services 2021. Inspection of Injectable Products for Visible Particulates. Guidance for Industry https://www.fda.gov/media/154868/download

[8] McKernan Substack 2024 https://anandamide.substack.com/p/vaccine-targeted-qpcr-of-cancer-cell

In May 2024, *König et al.*[9] detected DNA in BioNTech/Pfizer vaccine vials measuring 3,600-5,340 ng/dose, thus confirming results by *Speicher et al.* that residual DNA is also encapsulated within LNPs.

In a separate study of two BioNTech/Pfizer and one Moderna COVID-19 modRNA vaccine vials from Australia, *Speicher et al.* (September 2024)[10] issued an expert report for Australian legal proceedings documenting residual DNA levels up to 163 ng/dose for BioNTech/Pfizer and 8 ng/dose for Moderna when measured by qPCR, while the amount of total DNA as measured by Qubit® fluorometry was up to 848 ng/dose for BioNTech/Pfizer and 1,460 ng/dose for Moderna. This equated to 7-145 times above the 10 ng/dose regulatory safety threshold for non-encapsulated DNA.

In November 2024, *Raoult (2024)*[11] confirmed an excessive DNA content in the BioNTech/Pfizer product, including the presence of the SV40 promoter/enhancer within LNPs, up to an average of 5,160 ng/dose, being 516 times above the regulatory safety limit of 10 ng/dose. It was subsequently acknowledged that the methodology employed by Raoult should have included the application of RNase A, which would have reduced the estimated amounts by approximately 10-fold, to approximately 516 ng/dose, still approximately 51-fold above the regulatory safety limit.[12]

In December 2024, the peer-reviewed study by *Kämmerer et al.*[13] detected residual DNA levels 3-4 times above the regulatory limit in the BioNTech/Pfizer product, i.e. 2,712-3,683ng (after Triton-X-100 detergent treatment to disrupt LNPs) and 32.71–42.09ng (after Triton-X-100/RNase A treatment to additionally degrade RNA), with encapsulated DNA efficiently transfecting human HEK293 cells, including the SV40 promoter/enhancer sequence. It was further observed in an analysis by genomics expert McKernan of the *Kämmerer et al. (2024)* paper, that the readings of 32.71–42.09 ng/dose likely represented only 30% of the actual synthetic DNA present due to interactions with the fluorescent dye used for quantification, meaning the actual quantities of residual DNA were likely 40–133 ng/dose, or up to 133 times above the regulatory threshold.[14]

The study by *Ryan et al.* (2023)[15,] based upon 102 individuals from South Australian, revealed plasmid DNA sequences in blood samples from 75 individuals who had exclusively received the BioNTech/Pfizer or the Moderna products. Authors identified vaccine-derived DNA expression vectors, such as SV40 promoter/enhancer and kanamycin resistance gene sequences, which were not removed during the manufacturing process. Subsequent genomic analyses of the *Ryan et al.* (2023) data by *Chakraborty* (2024)[16] confirmed the presence of this DNA sequences in the blood of almost all 75 South Australian study participants. Further analyses by *McKernan* emphasized that

[9] *König et al.* 2024 https://doi.org/10.3390/mps7030041

[10] *Speicher* 2023, Expert report for use in the Australian Federal Court proceedings, https://www.dropbox.com/scl/fi/sb20elb520v6a1saxg9lj/240909-D-Speicher-Report.pdf; review by journalist Rebekah Barnett https://news.rebekahbarnett.com.au/p/breaking-dna-contamination-in-australian; review by former barrister Julian Gillespie https://julesonthebeach.substack.com/p/synthetic-dna-contamination-the-final

[11] *Raoult* 2024 https://hal.science/hal-04778576v1

[12] McKernan Substack 2024 https://anandamide.substack.com/p/more-papers-on-dna-contamination

[13] *Kämmerer et al.* 2024 https://publichealthpolicyjournal.com/biontech-rna-based-covid-19-injections-contain-large-amounts-of-residual-dna-including-an-sv40-promoter-enhancer-sequence/

[14] McKernan Substack 2024

[15] *Ryan et al.* 2023 https://doi.org/10.1016/j.xcrm.2023

[16] *Chakraborty* 2024 https://osf.io/preprints/osf/hzyn3_v1

the original detection methods were insufficient to identify longer and potentially more harmful DNA fragments, raising the likelihood that levels are far higher than initially reported.[17]

In December 2024, the peer-reviewed study by *Wang et al.*[18], supervised by FDA scientists and conducted within the FDA White Oak Labs, detected residual DNA levels 6-470 times above the regulatory threshold in BioNTech/Pfizer samples. Authors also demonstrated that residual plasmid DNA was replication-competent in *E. coli* following ligation and transformation.

Additional independent evidence has been reported by *Pékova* (Tilia Laboratories, Czech Republic)[19] in a quantitative multiplex real-time PCR analysis of 17 Moderna lots and 7 BioNTech/Pfizer lots. DNA-level spike protein cassette signals were detected in both products, with reported ranges of approximately 10^7–10^9 copies/ml for Spikevax and 10^8–10^9 copies/ml for Comirnaty. The author concludes that more detailed analysis is required for certain Moderna lots and, given the non-regulatory status of the report, these findings should be treated as requiring independent regulatory verification under a validated, reproducible protocol.

In September 2025, the peer-reviewed study by *Speicher et al.*[20] confirmed and expanded earlier analyses of Canadian vials. 32 COVID-19 modRNA vaccine vials from Ontario, Canada, representing 16 unique lots of BioNTech/Pfizer and Moderna products were analysed. Total DNA as measured by Qubit® fluorometry ranged 371–1,548 ng/dose for BioNTech/Pfizer and 1,130–6,280 ng/dose for Moderna. Specific plasmid DNA targets measured by qPCR were much lower. In BioNTech/Pfizer vials, 0.22–2.43 ng/dose for spike, 0.28–7.28 ng/dose for Ori, and 0.25–23.72 ng/dose for the SV40 promoter/enhancer-Ori. In Moderna vials, 0.25–0.78 ng/dose for spike and 0.01–0.34 ng/dose for Ori. The SV40 promoter/enhancer-Ori sequence was only detected in BioNTech/Pfizer vials. In addition, authors reported that total DNA levels exceeded the FDA and WHO regulatory threshold of 10 ng/dose in all vials tested when measured by fluorometry after accounting for non-specific binding to modRNA. The reported exceedance was 36–153 times for BioNTech/Pfizer and 112–627 times for Moderna. When measured by qPCR, all Moderna vials were within the regulatory limit, but 2 of 6 BioNTech/Pfizer lots, exceeded the 10 ng/dose threshold for the SV40 promoter/enhancer-Ori-target. This discrepancy is highly relevant as it reveals that the measured DNA amount strongly depends on the method used and that qPCR targeting selected plasmid targets cannot be treated as equivalent to a total DNA measurement in a fragmented modRNA vaccine product.

Authors also performed Oxford Nanopore long read sequencing on one vial and found mean and maximum DNA fragment lengths of 214 bp and 3.5 kb, respectively. This is important demonstrating that the detected DNA fragments were not limited to short fragments. The presence of DNA fragments above 200 bp and up to 3.5 kb supports the need for fragment-size analysis, sequencing, and product-specific assessment rather than reliance on a single qPCR assay.
The authors further reported that the detected DNA represented approximately 10^8 to 10^{11} plasmid DNA fragments per dose encapsulated in LNPs. This represents a major safety concern. Existing residual DNA limits were developed for naked DNA, but not for plasmid-derived DNA delivered in LNPs. The authors emphasised that LNPs increase transfection efficiency and that cumulative dosing presents significant and unquantified risks to human health.

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[17] McKernan Substack 2024 https://anandamide.substack.com/p/chakraborty-open-review; https://anandamide.substack.com/p/chakraborty-part-ii-odak-et-al; review by Dr. Demasi https://blog.maryannedemasi.com/p/part-1-blood-samples-contain-dna

[18] *Wang et al.* 2024 https://jhss.scholasticahq.com/article/127890-a-rapid-detection-method-of-replication-competent-plasmid-dna-from-covid-19-modRNA -vaccines-for-quality-control; review by McKernan https://anandamide.substack.com/p/fda-white-oak-lab-finds-6x-to-470x; review by Dr Demasi *https://blog.maryannedemasi.com/p/exclusive-fda-lab-uncovers-excess*

[19] *Pekova*, Tilia Laboratories (CZ) 2025 https://www.10letters.org/CzechResearch.pdf

[20] *Speicher et al.* 2025 https://doi.org/10.1080/08916934.2025.2551517

Finally, authors compared DNA loads with adverse events reported to VAERS and reported preliminary evidence of a dose-response relationship between DNA quantity per dose and the frequency of serious adverse events. This finding was not presented as proof of causation, but it is a pharmacovigilance signal that requires investigation. The appropriate safety response is independent lot-by-lot testing, publication of batch-level data, and further assessment of whether residual plasmid DNA load, SV40 promoter/enhancer-Ori sequences, LNP delivery, and cumulative exposure are associated with potential harm to human health.

Taken together, *Speicher et al*. shows that quantification of residual plasmid DNA in modRNA vaccine products is method- and lot-dependent and biologically relevant. The findings include high total DNA levels by fluorometry, lower target-specific qPCR values, SV40 promoter/enhancer-Ori detection only in BioNTech/Pfizer vials, DNA fragments up to 3.5 kb, up to 1.60×10^{11} plasmid DNA fragments per dose, and preliminary adverse-event dose-response findings. These results require transparent lot-by-lot final-product testing, orthogonal DNA quantification, fragment-size analysis, sequencing, LNP-related risk assessment, and human cell safety studies before the residual DNA issue can be considered resolved.

In March 2025, *Kaiser et al.* [21] published a paper arguing that earlier reports of excessive DNA impurities in BioNTech/Pfizer and Moderna products were caused by interference from high RNA and lipid concentrations. The authors used Qubit® fluorometry and LC-MS/MS and reported DNA:RNA mass ratios of approximately 1:1000, which they considered consistent with approved product specifications. The authors concluded that prior reports of DNA levels up to 534 times the regulatory threshold, as reported by *König et al.* (2024)[22], were incorrect and that residual DNA impurities in both BioNTech/Pfizer and Moderna vaccines remained within approved limits.

However, *Kaisers et al. c*riticism of the *König et al.* study is unconvincing since the authors own results clearly demonstrate that the measured amount of DNA depends on the analytical method used. In particular, the phenol/chloroform extraction and ethanol precipitation used to purify residual DNA from LNPs are not analytically neutral, as small DNA fragments may be preferentially lost during the process, resulting in under-quantification. Residual DNA quantification must be assessed in the final drug product using a method that is not affected by the sample preparation method. Furthermore, when there is a potential for RNA to interfere with DNA quantification, the correct approach is to remove or control for RNA without introducing a bias in DNA quantification. RNase treatment can be directly controlled for in the fluorometric assay, whereas post-RNase purification may result in the loss of smaller DNA fragments. Thus, the publication by Kaiser *et al.* provides further evidence that inappropriate preparation steps can lead to underestimation of residual DNA in LNP-formulated modRNA products. To ensure human health safety, all plasmid-derived DNA fragments in the final drug product, including small and long fragments, LNP-associated fragments, and biologically active sequence elements, need to be measured and characterised.

In December 2025, *Achs et al.*[23] published a study analysing 15 batches of the BioNTech/Pfizer and Moderna products available in Slovakia by qPCR, fluorometry, capillary electrophoresis and short-read sequencing. The authors concluded that residual DNA levels were below approved limits and consisted of small fragments derived from the DNA template used to produce modRNA . However,

[21] *Kaiser et al.* 2025 https://doi.org/10.1016/j.vaccine.2025.127022
[22] See note 9 above.
[23] *Achs et al.* 2025 https://doi.org/10.1038/s41541-025-01304-9

the conclusions of this study are incorrect for several methodological reasons, as analysed by McKernan in detail and summarized below[24]:

- First, *Achs et al.* treated vaccine samples with Triton X-100 prior to qPCR analysis but did not control for the efficiency by which Triton X-100 treatment alone released all LNP-associated nucleic acids. This is a major flaw. *McKernan et al. (2026)* [25] provided evidence that heating with Triton X-100 improves DNA detection substantially more than treatment with the detergent alone. When residual DNA is protected inside LNPs, then a room-temperature Triton X-100 treatment step cannot be assumed to lead to 100% recovery of residual DNA. Any preparation method that does not demonstrate complete LNP disruption and efficient DNA recovery prior to quantification cannot be used to assay residual DNA in the drug product accurately.

- Second, the DNA purification validation in *Achs et al.* is inadequate. The authors used spike-in DNA ladders to estimate residual DNA recovery, but this is inadequate unless recovery is quantified for all fragment sizes present in the vaccine being tested. A single value for aggregate recovery is not sufficient, as DNA purification methods are known to be size-dependent. Small DNA fragments can be lost during phenol-chloroform extraction, ethanol precipitation, silica column purification, or magnetic bead purification, while larger fragments may be efficiently recovered. Therefore, a reported overall recovery of 60–80% or 90–95% can still conceal poor recovery in certain fragment sizes, leading to underestimation of the total amount. Furthermore, size is not a determinant of biological activity – as is the case of the small but potent 72 bp SV40 promoter/enhancer fragment. Achs *et al.* used a 25–700 bp ladder for extraction recovery. This can be identified as a critical flaw because a 10–300 bp ladder was available and would have better tested the lowest fragment range. This matters because the authors' own argument depends on the claim that residual DNA is highly fragmented. If the fragments are claimed to be small, then the recovery control must prove recovery of very small fragments. A ladder that begins at 25 bp and is weighted toward larger, easier-to-recover fragments cannot validate loss across the full low-size range. It may overstate recovery and understate contamination.[25]

- Third, the purification chemistry itself may bias the result. LNPs, PEG, salts, RNase A, proteinase K, phenol-chloroform extraction, ethanol precipitation, silica surfaces and magnetic beads may all affect the efficiency of nucleic acid recovery. LNPs are not water-soluble and may interact with DNA and surfaces of tubes, pipette tips etc. Positively charged lipid components may bind or sequester negatively charged DNA. Excess protein from enzymatic treatment may also interfere with bead or column binding. Without matrix-matched spike-in controls across the relevant fragment-size range, the recovery of plasmid-derived DNA from the actual vaccine matrix remains unproven.[26]

- Fourth, the qPCR inhibition controls do not adequately establish that the vaccine matrix did not suppress amplification. The inhibition testing must be performed at the same effective DNA concentrations as the vaccine samples, not only at higher template concentrations where inhibition can be masked. The appropriate control is a serial dilution of the actual vaccine matrix showing the

[24] McKernan Substack https://anandamide.substack.com/, esp. "Could a Hidden Amyloidogenic Peptide in Pfizer's SV40 Promoter Contribute to Post-Vaccine Fibrin Clots? (21st August 2025); "Battles in Slovakia" (6th September 2025); "Pfizer's failure to linearize the vaccine plasmids" (3rd October 2025); "DAM methylation and cGAS-STING" (5th October 2025); "Cryptic Promoters" (8th October 2025); "Critical Review of Achs et al. (2025) on Residual DNA in modRNA Vaccine Preparations" (22nd October 2025); "RNA:DNA hybrids survive digestion in modRNA vaccine manufacturing" (5th December 2025); "A 2nd reason why the KAN gene is a problem" (8th December 2025); "DNA Purification vs Direct Measurement of Vaccine DNA" (18th December 2025); "More Holes in the Achs paper" (19th December 2025).

[25] See note 24 above, in particular: "DNA Purification vs Direct Measurement of Vaccine DNA" (18th December 2025).

[26] See note 24 above, in particular: "*DNA Purification vs Direct Measurement of Vaccine DNA" (*18th December 2025); "*Battles in Slovakia: micro-pipetting Vitriol" (*6th September 2025).

expected 3.3 Cycle threshold (Ct) shift for a ten-fold dilution. Without that, low qPCR values may reflect assay suppression rather than low DNA content. This is particularly important because modRNA, lipid particles, salts and formulation components can inhibit polymerase reactions or alter fluorescence.[27]

- Fifth, qPCR cannot measure total DNA amount in a fragmented plasmid mixture. It measures only the target region between the selected primer pair. Achs *et al.* used multiple primer sets, but the result still depends on amplicon length, target position, primer efficiency and template accessibility. Moreover, the use of longer amplicons, including 200 bp-plus targets, will undercount fragmented DNA compared with shorter assays such as for the 63 bp kanamycin resistance gene amplicon. This explains why different targets can give different results and why a single qPCR value cannot be used for a full product safety assessment.[28]

- Sixth, the primer design and target selection can also be called into question, as it does not properly match the Moderna reference sequence. If correct, this would further weaken any claim that the same assay design validly quantified both BioNTech/Pfizer and Moderna products. In a safety-critical product, primer sequences, reference sequences, amplicon lengths, conversion equations and raw Ct values must be transparently reported and independently testable.[29]

- Seventh*,* Achs *et al.* used short-read Illumina sequencing to infer the size distribution of residual DNA fragments. This method is not sufficient. Illumina sequencing measures the library that survived extraction, library preparation, adapter ligation, bead clean-up, PCR amplification and cluster generation. It does not directly measure the native DNA population in the vial. Shorter molecules amplify and cluster more efficiently. Longer molecules can be lost or underrepresented. Therefore, the finding of a median sequenced insert size of approximately 150 bp does not prove that longer plasmid fragments are absent. It only describes the fraction that survived that workflow and was sequenced efficiently.[30]

- Eighth, the Illumina library preparation itself can distort the result. Achs *et al.* used library amplification cycles and bead clean-up steps that enrich for shorter fragments and deplete longer or more difficult-to-amplify molecules. Omitting an enzymatic fragmentation step does not preserve the original fragment distribution when later steps still size-select the library. This is why long-read sequencing, such as Oxford Nanopore or PacBio, is required. Speicher *et al.* detected fragments up to 3.5 kb by Oxford Nanopore sequencing. Achs *et al.* did not use a method capable of excluding such a long-tail distribution.[31]

- Ninth, Achs *et al.* used a 300 bp emphasis in their discussion of fragment size, but the more relevant regulatory concern is DNA above 200 bp. *McKernan's* re-analysis argued that reads above 200 bp were substantially more frequent than those >300 bp reported in Achs *et al.* This is an important distinction. When the regulatory and biological concern begins only at approximately 200 bp, then presenting the data at a 300 bp threshold understates the relevant fraction of DNA fragments. Furthermore, the well-known biological activity of the 72bp SV40 promoter/enhancer element clearly demonstrates that fragments <200 bp may have potent effects.

- Tenth, the capillary electrophoresis data do not establish absence of DNA at the regulatory threshold. Achs *et al.* used Agilent Fragment Analyzer methods, including a high-sensitivity kit with a sensitivity range not adequate to exclude DNA at approximately 10 ng/dose when diluted across

[27] See note 24 above, in particular: "*Battles in Slovakia: micro-pipetting Vitriol" (*6th September 2025).
[28] Ibid.
[29] Ibid.
[30] Ibid.
[31] Ibid.

the vial volume and matrix. The 10 ng threshold corresponds to low pg/µL concentrations in a 300–500 µL vial. A method that is not sensitive enough at that concentration cannot be used to prove absence of DNA near the regulatory limit. It can only fail to detect a signal under its own detection threshold.

- Eleventh, the capillary electrophoresis method is also vulnerable to electrokinetic injection bias. Electrokinetic injection depends on charge, ionic strength, fragment size and matrix composition. Vaccine samples contain salts, lipids, PEG, enzymes, detergent and nucleic acid fragments. These components may alter injection efficiency and reduce the detection of larger or matrix-bound DNA fragments. Without matrix controls and internal standards, a weak or absent electropherogram signal is no evidence that the DNA is absent from the vial.

- Twelfth*,* Achs *et al.* did not address the full biological significance of bacterial plasmid-derived DNA. This DNA is not inert debris; it is bacterial plasmid DNA produced in *E. coli* and therefore may carry bacterial methylation patterns, bacterial backbone sequences, origins of replication, antibiotic resistance genes, and regulatory elements. *McKernan* separately argued that BioNTech/Pfizer plasmid DNA shows evidence of bacterial DNA methylation and incomplete linearisation in Oxford Nanopore sequencing. When plasmid-derived DNA is LNP-associated and delivered into human cells, then its degree and type of methylation, linearisation status, promoter activity and cellular localisation all impinge on safety.

- Thirteenth, the issue of incomplete plasmid linearisation is not resolved by *Achs et al.* According to McKernan's Oxford Nanopore analysis, reads spanning the *Eam*1104I restriction enzyme cutting site indicate incomplete linearisation of the circular plasmid DNA in Pfizer lots. This issue is relevant because circular or partially linearised plasmids are biologically distinct from short, linear DNA fragments. Sticky-end linear fragments may also circularize in cells. A safety analysis that does not directly assess circular DNA, nicked plasmids, sticky ends, fragment length and the potential for recircularization cannot be used to assess the risk of genotoxicity.

- Fourteenth, the possibility of cryptic mammalian promoter activity was not addressed. McKernan identified potential cryptic promoter activity in plasmid backbone regions, including the Ori region. This is relevant because one safety issue is whether plasmid-derived DNA can produce unintended transcripts following cellular delivery. When residual plasmid DNA enters human cells, cryptic promoter activity, read-through transcription and expression of adjacent sequences must be investigated experimentally by RNAseq and cell-based assays.

To summarize the shortcomings of the *Achs et al.* paper: The authors relied on methods that may result in the loss of DNA during extraction, suppress DNA amplification during qPCR, misrepresent DNA size during Illumina sequencing, and fail to detect DNA near the regulatory threshold by capillary electrophoresis. In addition, the authors did not establish complete LNP disruption, provide size-specific recovery data across the relevant fragment range, use long-read sequencing to exclude long plasmid fragments, test circular or incompletely linearised plasmid DNA, address cryptic promoter activity, or establish a safe threshold for LNP-encapsulated DNA.

The correct standard for human health protection is transparent lot-by-lot final-product testing using validated LNP disruption, heat-plus-detergent release controls, internal spike-ins, size-specific recovery ladders, qPCR serial dilution inhibition controls, multiple short and long amplicon targets, DNase/RNase-controlled fluorometry, matrix-controlled capillary electrophoresis, Oxford Nanopore or PacBio long-read sequencing, circular DNA assessment, methylation analysis, and human cell genotoxicity studies.

3 Potential Harm to Human Health

From a regulatory perspective, Comirnaty and Spikevax classify both as immunological and therefore biological medicinal products as well as under the gene therapy definition, but are excluded by way of legal derogation by the EU Commission from the applicability of gene therapy standards. For that, the protective purpose and objective of all EU pharmaceutical rules is to prevent harm to human health, which is the only appropriate post-marketing safety standard – if harm to human health arises, the medicinal product has to be withdrawn from the market. Therefore, the only relevant standard from a legal perspective is whether harm to human health through residual DNA could arise. Residual DNA is a biologically active process-related impurity and may pose a serious risk to human health that must be assessed in a product-specific manner.

As recognised in WHO TRS No. 978, Annex 3,[32] residual cellular DNA may raise safety concerns because of its potential infectivity and oncogenicity, and the FDA similarly explains that cell-substrate DNA may pose a risk where it encodes an infectious genome or a dominant activated oncogene capable of transforming recipient cells.

The oncogenic risk is not limited to the presence of a complete oncogene. WHO TRS No. 978, Annex 3, expressly requires that residual DNA be assessed not merely by reference to total mass, but also by reference to the quantity, size and biological activity of residual DNA fragments and the effectiveness of manufacturing steps intended to remove, fragment or inactivate DNA. This is consistent with Sheng-Fowler´s *et al.* [33] review of residual cell-substrate DNA in viral vaccines, which identifies oncogenicity and infectivity as the two principal biological activities of concern. It is also consistent with the broader literature on extracellular and circulating DNA, including Cheng *et al.,*[34] who discussed the possibility that circulating tumour DNA may be taken up by susceptible cells and contribute to oncogenic transformation.

This risk profile is altered for the worse when residual DNA is delivered by LNPs, specifically designed to deliver contained nucleic acids into the cell cytoplasm. In a Nature Communications article, Zhu *et al.*[35] demonstrated that LNPs can deliver plasmid DNA to cells in culture and *in vivo* and mediate transgene expression, including prolonged expression in animal models. Accordingly, residual plasmid DNA encapsulated by LNPs cannot be assessed in the same manner as naked DNA, as the formulation will increase cellular uptake and intracellular transport to the nucleus.

Residual DNA may also trigger innate immune activation. As Decout *et al.*[36] detail in their review on the cGAS–STING pathway, cytosolic DNA is a key trigger of innate inflammatory signalling, and inappropriate or persistent activation of this pathway is associated with inflammation, cellular stress, and tissue damage. Bacterial plasmid DNA may also contain unmethylated CpG motifs that can activate innate immune pathways, as described in the DNA vaccine literature on plasmid-backbone immunostimulation.

An additional safety concern arises where residual plasmid DNA contains bacterial backbone elements, including antibiotic-resistance genes. Plasmids are recognised vehicles of horizontal gene

[32] WHO, *Recommendations for the Evaluation of Animal Cell Cultures as Substrates for the Manufacture of Biological Medicinal Products and for the Characterization of Cell Banks*, Annex 3, in: *WHO Expert Committee on Biological Standardization, Sixty-first Report*, WHO Technical Report Series No. 978, Geneva: WHO, 2013, pp. 79–186.

[33] *Sheng-Fowler et al.* 2009 https://doi.org/10.1016/j.biologicals.2009.02.015

[34] *Cheng et al.* 2016 https://doi.org/10.18632/oncotarget.9453

[35] *Zhu et al.* 2022 https://doi.org/10.1038/s41467-022-31993-y

[36] *Decout et al.* 2021 https://doi.org/10.1038/s41577-021-00524-z

transfer in bacterial populations. Redondo-Salvo *et al.*[37] describe plasmids as mediators of horizontal transfer of antibiotic-resistance, virulence and other adaptive genes across bacterial populations. Although the probability of transfer to human microbiota after LNP injection requires product-specific assessment, the presence of such elements is not irrelevant and should be evaluated as part of the residual DNA risk assessment.

Another potential hazard to consider includes the risk of insertional mutagenesis following cellular uptake and nuclear access of residual DNA fragments, the persistence of episomal DNA leading to prolonged or unintended gene expression, and cumulative exposure from repeated dosing/boosting, which may increase the likelihood of biological events. Furthermore, residual DNA fragments containing regulatory elements or transcriptionally active sequences may exert biological effects even in the absence of full-length genes, particularly when delivered efficiently into cells by LNPs.

Finally, inadequate analytical control may itself create a safety risk. WHO TRS No. 978, Annex 3, cautions that the regulatory limit of 10 ng/dose does not consider critical factors such as DNA fragment size or DNA-inactivating steps, and further notes that different analytical methods may give different results for small fragments or DNA treated with inactivating agents. This concern is supported by the literature demonstrating that even very small DNA fragments may retain biological activity and may not be reliably detected by standard assays. For example, studies on DNA uptake and integration have shown that short DNA fragments can enter mammalian cells and participate in recombination or integration processes, as discussed by Folger *et al.* (1982)[38] and *Smithies et al.* (1985).[39] Further, research on circulating cell-free DNA, including work by *García-Olmo et al.* (2010)[40] and *Mittra et al.* (2015),[41] suggests that fragmented DNA can be taken up by recipient cells and may contribute to genomic alterations. These findings underscore that fragment size alone does not eliminate biological risk and analytical methods must be capable of detecting and characterising even very small DNA fragments to ensure an adequate safety assessment.

Therefore, reliance on qPCR alone is insufficient unless it is validated to detect total residual DNA, relevant fragment-size distributions, and sequence-specific DNA impurities of concern in the actual product matrix, including the final LNP-formulated drug product where appropriate. This concern is reinforced by *König et al.,*[42] who noted that qPCR is a method for quantifying specific DNA sequences, whereas assessment of total residual DNA and fragment-size distribution requires additional methodological consideration.

3.1 Relevant Regulatory Framework

The relevant regulatory framework required the EMA to assess residual DNA control through three central and interrelated guidelines of the 'International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use' (ICH).[43]

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[37] *Redondo-Salvo et al.* 2020 https://doi.org/10.1038/s41467-020-17278-2

[38] *Folger et al.* 1982 https://doi.org/10.1128/mcb.2.11.1372-1387.1982

[39] *Smithies et al.* 1985 https://doi.org/10.1038/317230a0

[40] *García-Olmo et al.* 2010 https://doi.org/10.1158/0008-5472.CAN-09-3513

[41] *Mittra et al.* 2015 https://doi.org/10.1007/s12038-015-9508-6 .

[42] See note 9 above.

[43] International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) develops internationally harmonized scientific and technical guidelines for pharmaceutical development and registration. In the European Union, finalized ICH guidelines are implemented by the EMA's Committee for Medicinal Products for Human Use (CHMP) as European scientific guidelines and form part of the regulatory framework applied in the assessment of medicinal products.

First, residual DNA had to be assessed as a process-related impurity requiring a product-specific acceptance criterion. Under ICH Q6B, specifications consist of tests, references to analytical procedures, and appropriate acceptance criteria. Process-related impurities, including DNA derived from the manufacturing process, must be controlled by scientifically justified and product-specific acceptance criteria and/or a control strategy.

Second, the EMA was required to assess whether the relevant CTD quality sections contained the necessary scientific and regulatory justification. Under the ICH M4Q structure, the quality dossier must, as applicable, include specifications, analytical procedures, validation of analytical procedures, batch analyses, impurity characterisation, and justification of specifications for the DS (drug substance) and/or the DP (drug product). Residual DNA control, therefore, had to be reflected in the approved dossier by demonstrating how the proposed limit, testing strategy, and manufacturing controls were justified for the specific product, given that modRNA technology is nascent and lacks mass-manufacturing experience.

Third, the EMA was required to assess the adequacy of the understanding of the manufacturing process, impurity clearance and the analytical control strategy. Under ICH Q11, process-related impurities must be addressed through a scientifically justified control strategy grounded in process understanding and the identification of relevant critical quality attributes. Under ICH Q2(R2), the analytical procedure used to quantify residual DNA, including qPCR or any alternative method, had to be validated and shown to be suitable for its intended purpose. This includes the method's ability to detect and quantify relevant residual DNA forms in the specific product matrix.

In summary, the EMA had to apply ICH Q6B, ICH M4Q, ICH Q11 and ICH Q2(R2) as the relevant regulatory framework for residual DNA control. These provisions required a product-specific assessment of residual DNA quantity, fragment-size distribution, biological activity, manufacturing clearance, analytical method suitability, batch consistency, and the specific formulation and delivery context of the modRNA -LNP product.

3.2 WHO TRS No. 978, Annex 3

In the CTD, specifically Module 3 (Quality), for the modRNA products Comirnaty and Spikevax, the WHO 10 ng/dose, as stipulated in WHO TRS No. 978, Annex 3, *Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks*, as a safe threshold for human health, was applied.
From a legal perspective, WHO TRS No. 978, Annex 3 has no binding legal force, but constitutes regulatory guidance for competent authorities, including the EMA.

From a substantive perspective, WHO TRS No. 978, Annex 3 applies only to animal cell cultures used as substrates and to the characterization of cell banks, and "specifically exclude[s] all products manufactured in embryonated eggs, microbial cells and plant cells" (WHO TRS No. 978, Annex 3, p. 89).

Moreover, WHO TRS No. 978 states clearly that the 10 ng per parenteral dose limit "does not take into consideration important factors such as the size of the DNA fragments and any potentially inactivating steps in the manufacturing process" (WHO TRS No. 978, Annex 3, p. 103).
The WHO recommendations further outline that the manufacturer should agree acceptable residual DNA limits for specific products with the NRA/NCL, considering the characteristics of the cell substrate, the intended use of the biological product and, most importantly, the effect of the manufacturing process on the size, quantity, and biological activity of residual DNA fragments (WHO TRS No. 978, Annex 3, p. 105). The reason for this is that the recommendations point to serious oncogenic concerns, particularly where residual DNA contains biologically active sequences

and where fragment size, sequence content, delivery context, and inactivation/removal have not been adequately characterized (WHO TRS No. 978, Annex 3, pp. 103–104).

Accordingly, the EMA acted in violation of the WHO recommendations by reducing their application solely to the numerical 10 ng/dose recommendation, despite the obvious exclusion of the new RNA-based genetic vaccination technology from their scope of application, and without further justification as to why this limit should otherwise apply.

Regarding the appropriate control method for total residual DNA levels and residual DNA fragment-size distribution, qPCR has been proposed. In any case, the WHO recommendations state that the method must be based on "sensitive and reliable assays for process- and product-related impurities," strict upper limits must be specified, and the method must be validated. It is further noted that "other methods may give different results for small fragments or for DNA that has been treated with inactivating agents" (WHO TRS No. 978, Annex 3, p. 106).

The WHO recommendations also explain why RNA is not considered a comparable risk factor, citing "the unstable nature of RNA and the lack of mechanisms for self-replication," but they expressly admit that "small non-coding RNA molecules – microRNA (miRNA) – that are more stable and have the capacity to modulate gene expression" may necessitate reassessment (WHO TRS No. 978, Annex 3, p. 106). This reasoning does not apply to the products at issue, as confirmed by the EMA's EPARs for Comirnaty and Spikevax, which explicitly describe the active substance as nucleoside-modified modRNA (modRNA), i.e. a methylpseudouridine-modified RNA (Comirnaty EPAR, p. 16; Spikevax EPAR, p. 15), i.e. artificially stabilised RNA elements.

In contrast to unmodified RNA, Comirnaty and Spikevax are products incorporating methylpseudouridine, which confer molecular stability, reduce innate immune recognition, and prolong intracellular persistence and translational activity. Furthermore, the modRNA is encapsulated in a novel LNP formulation specifically engineered to facilitate cellular uptake, endosomal escape, and cytosolic release of the modRNA, thereby enabling efficient interaction with the cellular translational machinery. This design inherently promotes sustained intracellular activity and may trigger downstream cellular responses, including cascades of gene expression and metabolic activation, which are not accounted for in the original assumption of rapid RNA degradation and therefore raise the possibility of carcinogenic effects, rendering the underlying assumptions of the recommendation inapplicable.

BioNTech/Pfizer and Moderna therefore lack any robust validation for applying the WHO recommendations on residual DNA control to a novel modRNA-LNP platform technology that has been explicitly excluded. From a regulatory perspective, the EMA violated its obligations and duties under Articles 12 and 14(1) of Regulation No 726/2004, which require that a marketing authorisation be granted only on the basis of a comprehensive scientific evaluation of the quality, safety and efficacy of the medicinal product, as well as under Article 8(3) of Directive 2001/83/EC, which obliges the applicant and the competent authority to ensure that the CTD dossier contains validated specifications, controls and acceptance criteria that ensure the products are safe and effective.

3.3 New Post-Authorisation Guidelines on the Quality Aspects of modRNA of WHO and EMA

The initial COVID-19 modRNA vaccines were authorised before dedicated modRNA -specific regulatory guidance was available. WHO subsequently adopted specific regulatory considerations for modRNA vaccines, published as Annex 3 to WHO Technical Report Series No. 1039 in 2022. WHO expressly recorded that the rapid authorisation and widespread use of COVID-19 modRNA vaccines

had created a pressing need for such guidance, while production and control methods were not yet standardised. It therefore required production and control procedures capable of affecting quality, safety or efficacy to be assessed with the competent national regulatory authority on an individual, case-by-case basis.[44]

The WHO guidance confirms a product-specific regulatory approach. Each vaccine must be evaluated on its own benefits and risks, and nonclinical evaluation must proceed on a product-specific basis. The linear DNA template is treated as the starting material for manufacture of the modRNA drug substance and requires control of its origin, sequence, manufacture, linearisation, removal and residual presence. The delivery system, including LNPs, likewise forms part of the assessment because formulation stabilises the modRNA , facilitates cellular uptake and may affect the quality, safety, immunogenicity and efficacy of the final product. Residual DNA template, enzymes, nucleotides, dsRNA, modRNA fragments and other process- or product-related impurities must therefore be controlled by sensitive and reliable analytical methods.[45]

The EMA subsequently developed dedicated EU quality guidance because the general guidelines for human vaccines did not specifically address modRNA technology. The EMA draft does not formally incorporate or cite the WHO guidance; it nevertheless converges with and further specifies the same product-specific control principles within the EU pharmaceutical framework.[46]
The current EMA draft Guideline on the quality aspects of modRNA vaccines[47] treats residual DNA template and host-cell DNA as process-related impurities requiring suitable and sufficiently sensitive analytical methods and a thorough risk assessment. It requires orthogonal methods to quantify and characterise residual DNA and expects fragment-size analysis to demonstrate the effectiveness of enzymatic digestion and purification. Specification limits must be clinically justified in accordance with ICH Q6B principles.

As a draft guideline, it is not yet binding law but its scientific requirements are nevertheless relevant to the continuing post-authorisation duty under Article 16(1) of Regulation No 726/2004 to take account of scientific and technical progress and, where required, to adapt manufacturing and control methods through an approved variation.[48]

3.4 Pharmacopoeia Texts

Moreover, the European Pharmacopoeia texts, which have been legally binding since 2026, confirm the requirement for product-specific residual DNA control. General Chapter 5.36, “modRNA vaccines for human use”; General Chapter 5.39, “modRNA substances for the production of modRNA vaccines for human use”; and General Chapter 5.40, “DNA templates for the preparation of modRNA substances”, establish a tailored regulatory framework for modRNA -LNP medicinal products, modRNA active substances, and the DNA template used as starting material.

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[44] WHO, *Evaluation of the quality, safety and efficacy of messenger RNA vaccines for the prevention of infectious diseases: regulatory considerations*, Annex 3, WHO Technical Report Series No. 1039 (2022).

[45] Ibid., pp. 89, 105–107, 112–119 and 131 (individual benefit-risk assessment; linear DNA template as starting material; control of DNA template and process-related impurities; product-specific nonclinical evaluation).

[46] European Medicines Agency (EMA), Draft Guideline on the quality aspects of modRNA vaccines, EMA/CHMP/BWP/82416/2025, 27 March 2025, pp. 3–4 and 15, esp. lines 56–64. The draft does not cite WHO TRS No. 1039 in its references; the relationship is substantive convergence rather than formal incorporation. https://www.ema.europa.eu/en/documents/scientific-guideline/draft-guideline-quality-aspects-modRNA -vaccines_en.pdf.

[47] EMA, Draft Guideline on the quality aspects of modRNA vaccines, EMA/CHMP/BWP/82416/2025.

[48] Ibid. sections 4.3–4.4, p. 6, lines 159–178 (residual DNA; sensitive and orthogonal methods; fragment-size analysis; ICH Q6B-based clinical justification of specification limits).

In particular, General Chapter 5.39 provides that residual DNA template is to be determined by quantitative PCR (qPCR) or digital PCR (dPCR), using a suitable detection method, and that the measured amount must comply with “the approved limit for the particular preparation”. It therefore presupposes prior regulatory assessment of the preparation, including the relevant manufacturing process, purification strategy, product matrix, analytical procedure and validation data.
At the same time, the wording of the Pharmacopoeia also confirms that a regulatory and methodological problem remains. Referring only to qPCR or dPCR as a “suitable detection method” does not define a fully harmonised measurement method, a uniform target sequence, a required extraction procedure, a method for distinguishing accessible from encapsulated or matrix-associated DNA, or a mandatory approach to fragment size distribution and biological activity. The suitability of the method, therefore, remains product- and process-dependent and must be demonstrated through validation, including specificity, sensitivity, recovery, limit of detection, limit of quantification, matrix interference, and the assay's ability to detect the relevant residual DNA species in the actual modRNA -LNP product.

Moreover, the requirement to demonstrate, during process validation, adequate removal of process-related impurities, including the DNA template, confirms that residual DNA control cannot be reduced to release testing alone. It requires evidence of manufacturing clearance, consistency across batches, and an analytically validated link between the measured signal and the actual residual DNA risk. General Chapter 5.40, in turn, establishes quality requirements for the DNA template itself as the starting material, but does not eliminate the need for a separate residual DNA control strategy for the modRNA substance and the finished modRNA vaccine product.
A similar regulatory rationale can be observed in recent United States Pharmacopeia and international developments. These standards focus on analytical control, characterisation, impurity assessment, and scientifically justified specifications, rather than prescribing a single, universally applicable residual DNA limit for all modRNA products. They also show that the exact analytical approach remains a live regulatory issue: qPCR and dPCR may quantify selected DNA targets, but the regulatory relevance of the result depends on primer/probe design, extraction and recovery, matrix effects, fragment-size distribution, reference standards, assay validation and the relationship between the measured target and total residual DNA.

4 Regulatory Failures

4.1 Post-Marketing Failure to Re-Assess the CTD Dossier

The EMA Draft Guideline on the quality aspects of modRNA vaccines, together with updated European Pharmacopoeia standards and WHO guidance, must be considered in their entirety when maintaining the marketing authorisation dossier.

Under the centralised procedure, applications for medicinal products for human use must include the particulars and documents required under Article 8(3) and Annex I to Directive 2001/83/EC, as incorporated by Article 6(1) of Regulation No 726/2004. The CHMP must verify that those documents comply with Directive 2001/83/EC and that the conditions for granting or maintaining the marketing authorisation are met.

After authorisation, Article 16(1) of Regulation No 726/2004 requires the marketing authorisation holder to take account of scientific and technical progress in respect of the methods of manufacture and control and to introduce any changes necessary to enable the medicinal product to be manufactured and checked by generally accepted scientific methods; Article 16(2) further requires the holder to provide the Agency, the Commission and the Member States with any new information which may entail amendment of the marketing-authorisation dossier. The same

obligation is reflected for human medicinal products in Article 23(1) and (2) of Directive 2001/83/EC.

Accordingly, where new scientific evidence, updated pharmacopeial standards, WHO guidance, or relevant regulatory guidance becomes available, including guidance specifically addressing modRNA vaccines, the EMA and the marketing authorisation holders are required to reassess and, where necessary, update the dossier, acceptance criteria, control strategy and validation methods accordingly, and failure to do so constitutes a post-marketing violation of the EMA's regulatory obligation.

4.2 Overview of Regulatory Failures in Residual DNA Control

- **Reduction to a numerical 10 ng/dose threshold**
  The EMA improperly reduced residual DNA control to the historical 10 ng/dose threshold without demonstrating that this limit was scientifically transferable to nucleoside-modified modRNA products as an LNP formulation. WHO TRS No. 978 generally links residual DNA safety to the manufacturing process, the size, quantity and biological activity of residual DNA fragments, and the validation of the analytical method used.

  The EMA failed to justify why the 10ng limit developed for conventional biological products could be applied without modification to a nucleic-acid product specifically designed for intracellular delivery.

- **Failure to perform a platform-specific modRNA-LNP risk assessment**

  The EMA failed to assess residual DNA in the specific context of the modRNA-LNP platform. Comirnaty and Spikevax are not conventional protein biologics but nucleic acid products manufactured from DNA templates and subsequently formulated into LNPs designed to protect nucleic acids, promote cellular uptake, enable endosomal escape, and permit intracellular release. This delivery context is scientifically relevant because residual DNA present in, associated with, or co-delivered by the LNP matrix may have a different recovery, detectability, and biological-availability profile than naked residual DNA in conventional biological products, as verified by Pardi *et al.*, 2018[49] and the Pfizer Residual DNA Characterization Report.[50]

- **Inapplicability of conventional RNA assumptions**

  The EMA failed to reassess its assumptions considering the instability of natural modRNA . WHO TRS No. 978 explains its reduced concern about modRNA by citing modRNA 's instability and the absence of self-replication mechanisms, while expressly noting that more stable small RNAs capable of modulating gene expression may require reassessment. That reasoning cannot be transposed without justification to methylpseudouridine-modified modRNA , which was developed to increase translation, reduce innate immune recognition, and improve biological stability, as described in *Karikó et al.*, 2008[51] and *Pardi et al.*, 2018.[52]

- **Failure to assess the administered finished product**
  The EMA accepted the residual DNA control primarily at the active substance level, although the product administered to patients is the finished modRNA-LNP drug product.

[49] See note 52 above.

[50] Pfizer Research and Development**,** *Residual DNA Characterization Report,* INX100594280: PF-07302048 (Comirnaty).

[51] *Karikó et al.* 2008 https://doi.org/10.1038/mt.2008.200

[52] *Pardi et al.* 2018 https://doi.org/10.1084/jem.20171450; https://doi.org/10.1038/nrd.2017.243

The EMA/Pfizer Residual DNA Characterization Report states that purified DS (drug substance) is further processed into DP (drug product) by combining modRNA DS with lipids to form LNPs. Residual DNA safety therefore requires either routine finished-product testing or validated bridging data demonstrating that active-substance testing reliably predicts finished-product exposure.
In addition, the regulatory purpose of distinguishing between drug-substance and drug-product testing is not only analytical but also supervisory: while drug-substance testing is typically performed by the manufacturer, finished-product testing enables independent verification by competent authorities such as the German Paul-Ehrlich-Institute (PEI). Such independent control is essential for ensuring product safety and regulatory credibility. Reliance solely on manufacturer-generated data, without systematic independent confirmation at the finished-product level, undermines the principle of external quality control and reduces the robustness of the overall safety assessment.

- **Insufficient DS/DP bridging**

  The EMA failed to require sufficiently validated DS/DP bridging data demonstrating that residual DNA measured in the DS reliably reflects residual DNA quantity, recovery, detectability, and biological relevance in the finished product. A mere assumption that no new DNA is introduced during DP formulation is insufficient, because the relevant question is not only whether DNA quantity increases, but whether the LNP formulation alters DNA recovery, assay performance, protection from degradation, cellular delivery, or biological presentation (EMA/Pfizer Residual DNA Characterization Report).

- **Failure to address LNP matrix effects**

  The EMA failed to verify how the LNP matrix affects DNA extraction, recovery, qPCR amplification, fluorescence-based total DNA measurement, and biological availability. LNPs are functional delivery systems, not passive excipients. They may alter nucleic acid accessibility, protect nucleic acids from degradation, interfere with analytical recovery, and affect whether residual DNA is available to cells. Therefore, matrix-specific validation was required before DS qPCR results could be considered sufficient for finished-product safety.[53]

- **Failure to validate LNP disruption and total DNA recovery**

  The EMA failed to require a finished-product method that demonstrates quantitative recovery of residual DNA after LNP disruption, including extraction, recovery, inhibition, and matrix controls. Fluorescence-based total-DNA methods following detergent disruption of LNPs have been proposed and analysed to quantify total DNA in the final product, whereas qPCR quantifies only a defined target sequence. The EMA therefore failed to require an orthogonal finished-product method capable of confirming the total residual DNA in the product administered to patients.[54]

- **Failure to verify DNase digestion robustness**

  The EMA failed to require and verify a robust DNase digestion step before authorisation. Residual DNA derives from the linear DNA template used in the in-vitro transcription reaction, and its clearance depends materially on DNase digestion and subsequent purification. The Comirnaty EPAR explicitly identified this as an unresolved issue, stating under Recommendation 7 that “additional studies are required to further demonstrate the

[53] See note 9 above.
[54] Ibid.

robustness of the DNase digestion step," thereby confirming that residual DNA clearance was a material process-control issue.[55]

- **Failure to correlate DNase activity with residual DNA clearance**

  The EMA failed to require a scientifically demonstrated correlation among DNase I activity, digestion efficiency, residual DNA quantity, and residual DNA fragment size distribution. Without such correlation, a qPCR value at the end of the process does not prove that the process consistently degrades and removes template DNA. A valid process-control strategy therefore requires evidence linking enzymatic activity, digestion conditions, fragment-size outcome, purification clearance and residual DNA release results.[56]

- **Failure to verify the purification clearance of all relevant DNA species**

  The EMA failed to verify that purification removed not only intact template DNA, but also fragmented, partially digested, chemically altered, template-associated or otherwise biologically relevant DNA species. DNase digestion creates smaller DNA fragments and oligonucleotides; purification must therefore be assessed not merely by disappearance of one qPCR amplicon, but by whether all relevant DNA species are removed or reduced to justified levels, as clearly described in Moderna's patent[57] and acknowledged in the EMA Draft Guideline.[58]

- **Failure to characterise the residual DNA fragment size distribution**

  The EMA failed to require adequate routine characterisation of the residual DNA fragment size distribution. Fragment size is central to residual DNA risk assessment because it determines qPCR detectability, persistence of functional sequence elements, potential biological activity, and whether DNase digestion and purification achieved their intended purpose. WHO TRS No. 978[59] expressly recognises that different methods may give different results for small fragments or DNA treated with inactivating agents; the EMA's later modRNA draft guideline likewise expects fragment-size analysis to demonstrate enzymatic and purification effectiveness (WHO TRS No. 978, Annex 3; EMA/CHMP/BWP/82416/2025).

- **Failure to assess biologically relevant fragmented DNA**

  The EMA failed to assess whether fragmented residual DNA could remain biologically relevant despite reduced qPCR detectability. Preclinical evidence by Raghuram *et al.*, 2017[60] and Lim *et al.*, 2023[61] indicates that, in model systems, physically shared DNA fragments can enter cells and cell nuclei under conditions in which high-molecular-weight DNA did not, and that linear DNA introduced into mammalian cells by transfection can undergo stable chromosomal integration. These findings demonstrate why residual-DNA

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[55] EMA*, Comirnaty: Public Assessment Report*, EMA/707383/2020, 21 December 2020, section 2.2.6, "Recommendations for future quality development", Recommendation 7, p. 40: "The MAH should provide the results of the studies performed to enhance the robustness of the DNase digestion step." The assessment further states that the robustness of the DNase digestion step had not been comprehensively demonstrated and required additional validation data.

[56] Ibid., see also EMA, Draft Guideline on the quality aspects of modRNA vaccines, note 47 above, pp. 6–7 (residual DNA control, orthogonal methods, fragment-size analysis and demonstration of DNase/purification effectiveness.

[57] Issa et al., Removal of DNA Fragments in modRNA Production Process, US Patent No. US 10,077,439 B2**,** assigned to ModernaTX, Inc.; EMA Draft Guideline EMA/CHMP/BWP/82416/2025.

[58] EMA Draft Guideline EMA/CHMP/BWP/82416/2025, see note 47 above.

[59] See note 44 above.

[60] *Raghuram et al.* 2017 https://doi.org/10.1186/s12867-017-0098-8

[61] *Lim et al.* 2023 https://doi.org/10.1038/s41598-023-33862-0

fragment size, delivery context, and biological activity could not be dismissed without product-specific assessment.

- **Overreliance on qPCR as a surrogate for total residual DNA**

  The EMA accepted qPCR as the method for controlling residual DNA without adequately addressing its target specificity, which means it does not quantify total residual DNA. qPCR measures only DNA molecules containing the selected primer-binding sites and an intact amplicon. It cannot quantify fragments outside the target region, fragments shorter than the amplicon, fragments lacking one primer site, or DNA species whose recovery is impaired by the product matrix. Therefore, treating qPCR compliance as equivalent to total-DNA control was scientifically insufficient, as described in König *et al.*[62] and Moderna's patent.[63]

- **Failure to address qPCR amplicon coverage and mathematical extrapolation**

  The EMA failed to disclose and justify the exact qPCR amplicon, primer location, amplicon length, target coverage, and the representativeness of the full plasmid/template impurity profile. When a short qPCR amplicon covers only a small fraction of the original DNA template, compliance with a mass limit necessarily depends on mathematical extrapolation from a sequence-specific signal. This creates a regulatory vulnerability: the selected target region can materially determine the reported residual DNA value, and without transparent disclosure and validation, the EMA could not verify that the chosen amplicon was representative.[64]

- **Failure to exclude assay design bias**

  The EMA failed to disclose and verify whether the BioNTech/Pfizer and EMA qPCR assays used in internal and external controls relied on a single amplicon, whether adequate internal controls were used, and whether primer selection could bias the result toward regulatory compliance. The regulatory failure consists in the fact that the assay strategy, including the precise design, target coverage, internal controls, extraction recovery, and matrix performance, is not approved by EMA for which the precise design, target coverage, internal controls, extraction recovery, and matrix performance were not transparently demonstrated in the public record.[65]

- **Failure to assess spike-region and RNA-associated residual DNA**

  The EMA failed to require experimental verification that all plasmid/template regions, including the spike-coding region, were equally accessible to DNase digestion, purification, and qPCR detection. If RNA hybridisation or template/transcript association reduces DNase accessibility and activity in specific regions, and the qPCR assay does not target those regions, residual DNA may be materially underestimated. Speicher *et al.* reported that different qPCR amplicons yielded markedly different residual DNA estimates, illustrating why target selection can substantially affect quantification and why a single-amplicon strategy is insufficient without orthogonal confirmation, as outlined in *Speicher et al.*[66]

- **Failure to resolve qPCR/fluorometry discrepancies**

  The EMA failed to address the scientific significance of the divergent residual DNA values obtained by qPCR and total DNA methods. EMA authorised BioNTech/Pfizer to quantify residual DNA by using a qPCR control method at the active substance with no further

[62] See note 9 above.
[63] See note 58 above.
[64] See note 9 above.
[65] Ibid., with explicit reference to note 9.
[66] See note 20 above.

control methods, particularly no fluorescence spectroscopy at the level of finished product release testing (batch testing).[67]
BioNTech/Pfizer measures DNA impurities in the active substance using qPCR, with a target of less than 1% of the original DNA template. This indirect approach estimates compliance with contamination limits via mathematical extrapolation. Indeed, the qPCR sequence can be easily adapted by BioNTech/Pfizer to meet regulatory safety acceptance criteria. Without disclosing the exact qPCR assay used by BioNTech/Pfizer and the EMA respectively in their internal and external controls to quantify residual DNA, one could assume that only a single amplicon without an internal control was applied to meet the acceptance criteria.

For example, the DNA encoding the spike sequence has modRNA bound to it that inhibits the enzyme DNase I from processing and removing this DNA. Consequently, if the qPCR assay used by EMA or BioNTech/Pfizer doesn't include the spike region of the plasmid, the results would be 100-fold off in their estimates. This has also been demonstrated by Speicher *et al.*, that the use of two qPCR amplicons manifests in a 100-fold difference in quantitation of residual DNA.

The methodological considerations of the qPCR versus fluorescence spectroscopic methods in the quantification of residual DNA have been thoroughly analysed by *König et al.* A control method that quantifies total DNA impurities in the final product would be possible by dissolving LNPs with a detergent, as done by fluorescence spectroscopy methods. Experimental testing of this approach confirms that reliable values of total residual DNA can be obtained in this way.
Moreover, the legally binding European Pharmacopoeia (Ph. Eur.) control methods for quantifying residual host cell DNA also state that, although qPCR is the method of choice for quantifying specific DNA sequences, the measurement of total DNA is assigned to other methods.[68i] The Moderna Patent on "[r]emoval of DNA fragments in modRNA production process" confirms also that "[q]uantitative PCR is often applied to measure the residual DNA but it only detects the DNA molecules that contain both qPCR primers thus does not measure all other smaller DNA molecules that are partially digested. To overcome this challenge, a liquid chromatography-tandem mass spectrometry (LC/MS/MS) approach can be used where a total nuclease digestion is performed on the RNA drug substance sample following the DNA removal step."[69]

- **Failure to require an orthogonal total-DNA control strategy**

  The EMA failed to require an orthogonal total-DNA control strategy. The European Pharmacopoeia distinguishes sequence-specific DNA quantification from total-DNA measurement: qPCR can quantify a defined target sequence, but it cannot, by itself, establish total residual-DNA control.[70] This is critical for modRNA products because partially digested DNA fragments, off-target template regions, short DNA species and matrix-associated DNA may escape a single qPCR amplicon. Moderna's own patent confirms that qPCR does not measure smaller partially digested DNA molecules lacking both primer sites and identifies LC/MS/MS after total nuclease digestion as a possible total-DNA approach.[71]

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[67] EMA, European Public Assessment report, EMA/707383/2020, p.21, available at https://www.ema.europa.eu/en/documents/assessment-report/comirnaty-epar-public-assessment-report_en.pdf.

[68] Ph. Eur., section 2.6.35 'Quantification and Characterization of Residual Host-Cell DNA', reprinted, Free access to supportive pharmacopoeial texts in the field of vaccines for human use during the coronavirus disease (COVID-19) pandemic, October 2020, https://www.edqm.eu/en/d/99080?p_l_back_url=%2Fen%2Fsearch%3Fq%3Dsv40

[69] See note 58 above. United States Patent, IIssa et al. (Moderna), Patent No.: US 10,077,439 B2, available at

[70] See note 68 above.

[71] See note 57 above.

EMA therefore should have required LC/MS/MS, fluorometry or an equivalent validated orthogonal method to verify total residual DNA and fragment-size distribution in the finished LNP product, as described in König et al.[72]

- **Failure to require full plasmid/template annotation**

  The EMA failed to require complete disclosure, annotation, and risk assessment of all plasmid/template elements relevant to residual DNA safety, including non-coding and regulatory elements. Residual DNA risk depends not only on mass but also on sequence identity. Promoters, enhancers, origins of replication, antibiotic-resistance markers, open reading frames, polyadenylation signals and other functional elements may be biologically relevant even at low total mass. The EMA therefore failed to require a sequence-specific impurity assessment according to their own Draft Guideline.[73]

- **Failure to assess sequence-specific impurities and regulatory DNA elements**

  The EMA treated residual DNA as a generic mass-based impurity, although biological risk also depends on sequence identity and regulatory function. A residual DNA assessment therefore had to address promoter, enhancer, origin-of-replication, antibiotic-resistance, open-reading-frame and other functional or potentially functional plasmid-derived elements. A mass-based qPCR value cannot indicate whether such elements persist, whether they are detectable by the chosen assay, or whether they require specific toxicological assessment, as confirmed in WHO TRS No. 978, Annex 3[74] and the Draft Guideline.[75]

- **Failure to integrate residual DNA into the full CMC control strategy**

  The EMA failed to integrate residual DNA control into a coherent CMC strategy covering starting material, IVT, DNase digestion, purification, analytical validation, active-substance release, finished-product testing, stability, batch consistency and biological safety. Residual DNA is not an isolated impurity; it is linked to the DNA template, the IVT process, enzyme performance, purification robustness, method selection, LNP formulation and the product administered to patients, as evident from Comirnaty's EPAR[76] and the Pfizer Residual DNA Characterization Report.[77]

- **Failure to reassess Process 1/Process 2 comparability**

  The EMA failed to ensure that clinical Process 1 material and commercial Process 2 material were fully comparable with respect to DNA-template manufacture, purification, residual DNA impurity profile, modRNA integrity, and other relevant critical quality attributes. The Comirnaty EPAR records that efficacy, safety and immunogenicity were demonstrated using clinical batches from Process 1, while commercial batches were produced using a different Process 2, requiring comparability of the active substance and finished product. Residual DNA control should therefore have been specifically included in the comparability assessment.[78]

- **Failure to ensure commercial scale and site-to-site consistency**

  The EMA failed to require sufficient evidence that residual DNA clearance was consistent across commercial-scale batches, manufacturing sites, enzyme lots, and process changes.

[72] See note note 9 above.
[73] See note 47 above.
[74] See note 32 above.
[75] See note 47 above.
[76] See note 46 above.
[77] See note 50 above.
[78] See note 55 above.

The Pfizer Residual DNA Characterization Report[79] evaluated drug-substance batches from multiple commercial manufacturing sites and variants, confirming that site-to-site and batch-to-batch consistency was a relevant regulatory issue. Such consistency could not be established merely by a single qPCR specification without broader process and method validation.

- **Failure to assess innate immune and biological-activity risks**
  The EMA failed to assess whether residual DNA species could trigger innate immune pathways or other biological responses in the actual LNP delivery context. Cytosolic DNA is biologically active and can activate cGAS-STING DNA-sensing pathways. This concern is supported by *Yu et al.* (2021), who show that cGAS–STING signaling can strongly modulate innate antiviral immunity. These findings emphasize that residual DNA in nucleic-acid delivery products may pose a biological hazard and should therefore be evaluated for immunostimulatory activity, not merely quantified by mass.[80]

  Although modRNA vaccines are legally excluded from the gene-therapy category of Advanced Therapy Medicinal Products (ATMPs),[81] their mechanism engages the same underlying risk logic: ATMPs comprise gene-, cell- and tissue-based medicinal products whose biological activity requires specific assessment of intended and unintended effects arising from the product and its manufacturing process. Accordingly, EMA was required to assess not only the intended antigen expression, but also unintended biological activity arising from product-related or process-related nucleic-acid species. Annex I, Part IV of Directive 2001/83/EC and the EMA/CAT risk-based approach require product-specific risk profiling where risks derive from quality attributes, biological activity, non-cellular components, biodistribution, manufacturing impurities or contaminants, including unwanted immunogenicity and toxicity. Residual DNA in an LNP-formulated nucleic-acid product therefore had to be assessed as a potentially bioactive impurity capable of triggering innate DNA-sensing pathways, including cGAS–STING, rather than being controlled only by a mass limit. EMA's own SO1 confirms the same regulatory logic: EMA required further characterisation of truncated and modified modRNA species and their potential translation into unintended proteins or peptides, including homology with human proteins that could cause autoimmune processes. However, SO1 remained too narrow for the residual-DNA problem: it addressed autoimmune risk from unintended translated proteins/peptides, but not innate immune activation by residual DNA itself, not cGAS–STING biological activity, not LNP-mediated cellular delivery of DNA species, and not downstream inflammatory or antiviral pathway disturbance. Under the ATMP framework, EMA therefore had to require product-specific testing of residual-DNA biological activity, cellular uptake, biodistribution, persistence, innate immune activation, cytokine/IFN signaling, and interaction with nucleic-acid sensing pathways before dismissing the risk.

- **Failure to assess integration-related risk in an LNP-formulated context**
  The EMA failed to assess the residual DNA integration-related risk in the actual modRNA-LNP delivery context. The relevant question was not whether integration was clinically proven, but whether fragmented or linear residual DNA, delivered or protected by an LNP-formulated product, required risk assessment for nuclear exposure, DNA repair interactions, episomal persistence, or low-frequency integration. Experimental evidence by Raghuram *et al.*, 2017[82] and Lim *et al.*, 2023[83] on sheared DNA uptake and linear DNA integration demonstrates why this question could not be dismissed without assessment.

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[79] See note 77 above.
[80] *Yu et al.* 2021 https://doi.org/10.1080/19490976.2021.1959839
[81] Directive 2001/83/EC, Annex I, Part IV, section 2.1.
[82] *Raghuram et al.* 2017 https://doi.org/10.1186/s12867-017-0098-8.
[83] *Lim et al.* 2023 https://doi.org/10.1038/s41598-023-33862-0.

Annex I, Part IV of Directive 2001/83/EC expressly identifies “the level of integration of nucleic acids sequences or genes into the genome” and “the risk of oncogenicity” as legally relevant risk factors in the ATMP risk analysis. Therefore, where fragmented or linear residual DNA is present in a nucleic-acid product and is delivered or protected by an LNP system, EMA had to require product-specific data on cellular uptake, biodistribution, persistence, nuclear exposure, episomal persistence, DNA-repair interaction, genotoxicity, insertional-mutagenesis potential and integration-site analysis where indicated. If EMA considered such testing unnecessary, it had to provide a scientific justification in Module 2.

- **Failure to assess cumulative exposure**

  The EMA failed to assess residual DNA exposure in the context of repeated dosing, boosters, variant-updated products, vulnerable groups, and population-wide administration. A per-dose threshold does not, by itself, address cumulative exposure or repeated administration, particularly when the product platform is designed for intracellular nucleic acid delivery and residual DNA may be carried forward into the finished LNP product.

- **Failure to reassess the dossier after new scientific evidence and guidance**

  The EMA failed to ensure that the marketing-authorisation dossier, specifications, and control methods were reassessed in light of evolving scientific evidence and modRNA - specific regulatory guidance. The Draft Guideline[84] now expressly requires orthogonal residual DNA methods and fragment-size analysis to demonstrate the effectiveness of enzymatic and purification processes. That guidance confirms, at a minimum, that residual DNA control for modRNA vaccines requires a more specific analytical strategy than reliance on a single qPCR-based active substance assay.

In summary, the EMA failed to provide a transparent, independently verifiable justification for residual DNA acceptance criteria, assay selection, amplicon design, target coverage, reference standards, recovery controls, matrix validation, DS/DP bridging, fragment-size analysis, and finished-product safety. Without disclosure of the exact assay design and validation logic, the EMA’s conclusion that residual DNA was adequately controlled cannot be independently verified.

5 Conclusion: Stop, Unredact and Re-evaluate

The evidence set out in this report is sufficient to establish an overriding public interest in disclosure under Article 4(2) of Regulation No 1049/2001. Transparent disclosure is necessary to permit an independent scientific and regulatory verification of residual DNA control, including the validity of the qPCR method, the adequacy of the control strategy, and compliance with applicable quality and safety requirements and explicitly required under the precautionary principle.

This interest is particularly compelling because Comirnaty and Spikevax are prophylactic medicinal products administered on a population scale. Their continued authorisation therefore requires the highest standards of public-health protection, regulatory accountability and scientific verifiability. These objectives lie at the core of Regulation No 1049/2001 and EU pharmaceutical law.

The requested documents are necessary to verify whether the authorised products comply with Union requirements. Commercial confidentiality cannot prevail where withholding the underlying analytical procedures, validation data, batch results, plasmid characterisation, finished-product assessments and regulatory reasoning prevents such verification. Confidential assertions of compliance cannot substitute for access to the evidence on which compliance was established.

[84] See note 58 above.

Accordingly, any reliance on the commercial-interests exception under Article 4(2) of Regulation No 1049/2001 must yield to the overriding public interest in disclosure. The present request identifies specific unresolved quality and safety concerns, the precise documents required to assess them, and the direct necessity of disclosure for independent verification.

The unresolved concerns regarding residual DNA, analytical validity and product-specific control further require EMA and the Commission to reassess whether the conditions for maintaining the marketing authorisations remain fulfilled. In light of the evidence, appropriate regulatory measures, including variation, suspension or revocation under Article 116 of Directive 2001/83/EC and, where applicable, the procedure under Article 20 of Regulation No 726/2004, must be implemented.

The marketing authorisations cannot lawfully be maintained while the underlying quality and safety data are withheld as commercially confidential information, thereby precluding independent scrutiny and scientific reassessment of their safety implications.