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ALC-0315 Toxic Metabolites: Pharmacokinetic and Regulatory Criticalities in a COVID-19 "mRNA Vaccine"

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Abstract

ALC-0315, the ionizable cationic lipid enabling the Pfizer vaccine Comirnaty's LNP platform, is presented in regulatory files as if its metabolic fate were straightforward and well controlled. Our analysis instead identifies a basic, consequential discrepancy that should never survive dossier assembly — let alone review: the hydrolysis product dictated by the ALC-0315 chemical structure is *2-hexyldecanoic acid* (α-branched), yet key submissions by Pfizer repeatedly invoke the presence of distinct *6-hexyldecanoic* isomer (not commercially available, not recognized as an analytical standard in established analytical protocols). This is a material misidentification with direct safety and compliance consequences. The authentic metabolite, 2-hexyldecanoic acid, concealed by Pfizer and disguised as 6-hexyldecanoic acid, carries an *H410* classification (*very toxic to aquatic life with long-lasting effects*), while the "phantom" 6-hexyl isomer is lacking any hazardous classification and presented as theoretically more degradable. In a CMA supporting pivotal preclinical study, substituting 2-hexyl with an unclassified 6-hexyl isomer materially compromises the clearance account, traceability, and any defensible claim of GLP grade validation for Pfizer's vaccine. The second breakdown product (a trialkanolamine, pKa ≈ 9.6) is liable to lysosomal sequestration, thereby impeding clearance; however, the company dossier offers only limited substantiation of its disposition. Critically, the pharmacokinetic dataset does not adequately account for dose biodistribution and is predicated on insufficiently documented — or omitted — testing standards. Given the scale of exposure (billions of subjects) and the potential oncological and cardiological consequences placed on these PK criticalities, these omissions are not merely unfortunate; they are utterly unacceptable. Accordingly, we call for an independent, methodologically transparent and fully auditable re-assessment of ALC 0315 metabolism and clearance, and for all regulatory decisions concerning the Comirnaty medicinal product to be reconsidered in view of what appears to amount to a grave and consequential omission in verification procedures.

Keywords: *ALC-0315, ionizable lipid, lipid nanoparticles, LNP, COVID-19, mRNA vaccine, ribosomal frameshifting.*

1. INTRODUCTION

Pharmacokinetic (PK) studies, particularly those involving laboratory animal models, are essential and mandatory prerequisites for obtaining marketing authorization (MA) for any novel pharmaceutical or vaccine. They constitute the cornerstone of the pre-clinical phase, facilitating the delineation of the

safety profile and the prediction of the physiological behavior of the formulation's constituents within the human organism. Specifically, the metabolic analysis of both active pharmaceutical ingredients (APIs) and excipients in animal models is critical, as it enables researchers to determine whether the biological system transforms the agent into active, inactive, or toxic metabolites prior to the commencement of human clinical trials.

For medicinal products intended for human use, the primary regulatory frameworks in Europe include:

1. Directive 2001/83/EC: The foundational legislative text for human medicines, establishing the obligation to submit a comprehensive dossier of pharmaco-toxicological evidence (Annex I) as a prerequisite for securing an MA.
2. Regulation (EC) No 726/2004: Governing the community procedures for the authorization and supervision of medicinal products and establishing the European Medicines Agency (EMA).

ALC-0315 (Chemical Name: [(4-hydroxybutyl)azanediyl]di(hexane-6,1-diyl) bis(2-hexyldecanoate); CAS No. 2036272-55-4) represents a foundational constituent of the Pfizer/BioNTech Comirnaty vaccine, playing a critical role in the endosomal internalization of lipid nanoparticles (LNPs) and the subsequent cytosolic translocation of mRNA following intramuscular administration (EMA, 2021).
The apparent pKa of ALC-0315 (6.09) is inherently suboptimal for intramuscular application and efficient transfection (Hassett et al., 2019; Segalla, 2023b), diverging from the conclusions reported by the EMA (2021, p. 42). Its intrinsic pKa (9.6) mandates a high degree of cytosolic protonation, which is correlated with the induction of pro-inflammatory cytokines and the generation of reactive oxygen species (ROS) (Yu et al., 2020; Patel et al., 2021; Ndeupen et al., 2021; Segalla, 2023a, 2023b, 2024). Moreover, ALC-0315 is not included in the European Pharmacopoeia and does not appear in the EU C&L (classification and labelling) inventory. As a consequence, the substance has no identifiable REACH registration number and no publicly traceable CLP classification. In practical terms, this means that its overall toxicological profile is not officially established — neither for the substance as such, nor for its nanoforms within lipid nanoparticles (Segalla, 2023a).

This critical review focuses on the analysis of the pharmacokinetic and metabolic studies submitted by Pfizer to the EMA in August 2020, aimed at securing the conditional marketing authorization granted on December 21, 2020. Such studies, conducted by Pfizer and subsequently endorsed by the EMA and FDA, are detailed in the report titled *Investigation of the biotransformation of ALC-0159 and ALC-0315 in vitro and in vivo in rats* (Pfizer 043725, 2020), which provides a preliminary qualitative assessment of the biotransformation of ALC-0159 and ALC-0315 within systemic circulation, hepatic S9 fractions, and hepatocytes across mouse, rat, monkey, and human models, as well as in plasma, urine, feces, and liver samples derived from rat-based pharmacokinetic assays.

This review focuses on the chemical, biochemical, and regulatory inconsistencies that emerge from a critical appraisal of Pfizer's UHPLC–MS/MS data on metabolites of the ionizable cationic lipid ALC-0315.

The technique known as UHPLC-MS/MS (Ultra-High Performance Liquid Chromatography-Tandem Mass Spectrometry) combines rapid, high-resolution liquid chromatography with sensitive tandem mass spectrometry to identify and quantify compounds in pharmaceuticals, environmental monitoring, metabolomics, and clinical diagnostics. It efficiently separates complex samples and detects specific

analytes, providing detailed structural data and accurate quantification even at very low concentrations, of the order of picograms (Sanjeev et al., 2022; Khan, 2024)

2. THE "PHANTOM STANDARD" DECEPTION: DOCUMENTARY FALSIFICATION AND CONCEALMENT OF THE TOXIC CATABOLITE 2-HEXYLDECANOIC ACID

According to Pfizer, UHPLC-MS/MS mass spectrometric analyses indicate that the metabolism of ALC-0315 proceeds primarily through two sequential stages of ester hydrolysis, yielding a monoester

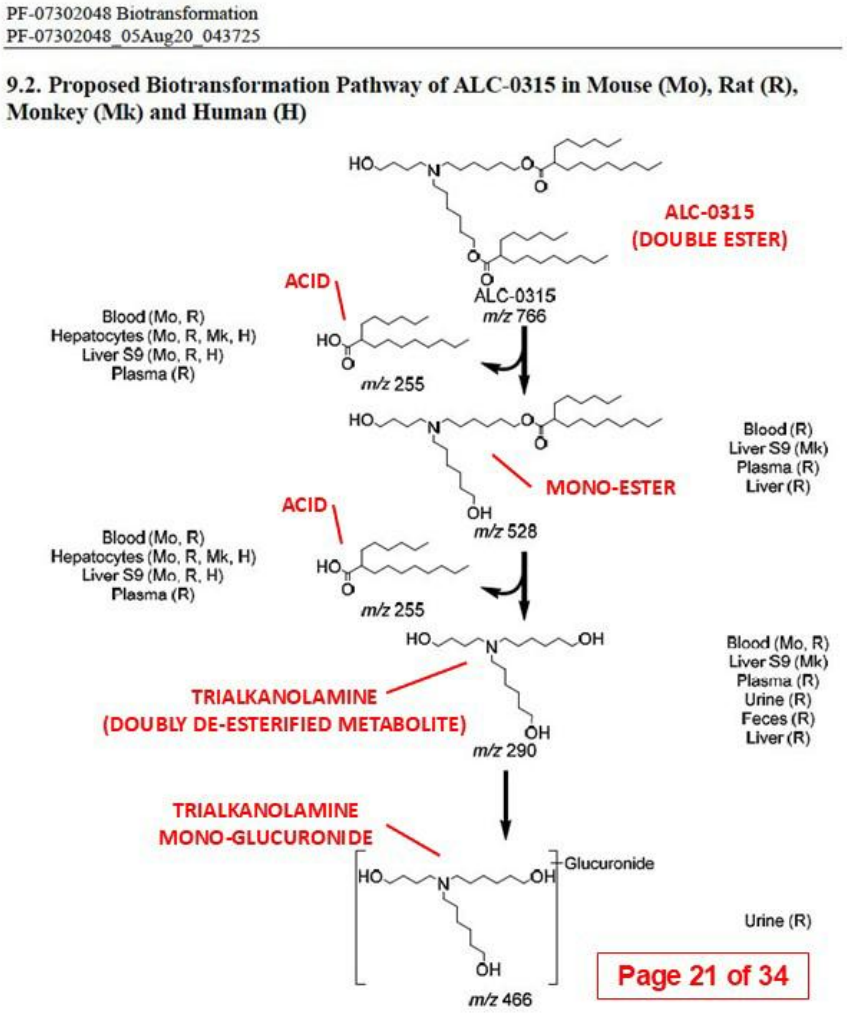
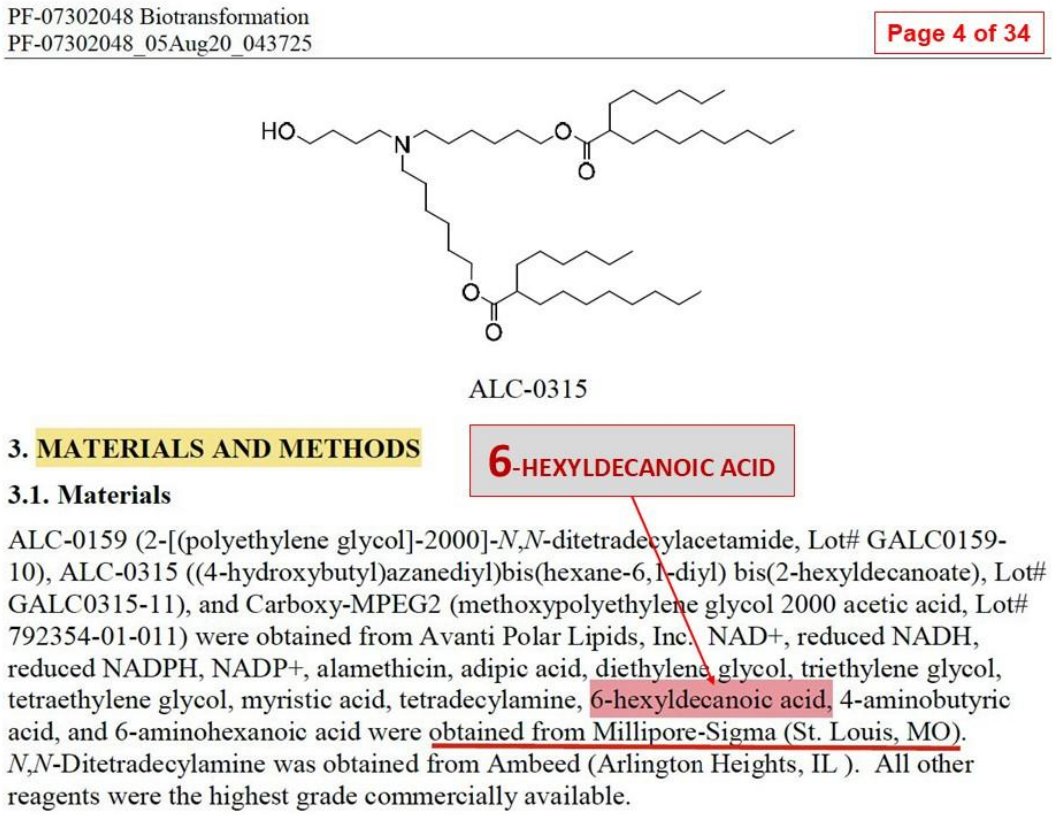


Figure 1. Schematic overview of the metabolic biotransformation pathway of ALC-0315 according to Pfizer Report PF-07302048_05Aug20_043725.

intermediate (mass-to-charge ratio m/z: 528) and a doubly de-esterified metabolite (m/z: 290). The monoester was identified in rat blood, plasma, and liver, as well as in monkey S9 fractions; conversely, the doubly de-esterified metabolite was detected in mouse and rat blood, monkey liver S9 fractions, and plasma, urine, feces, and liver from rat tissues and fluids. This metabolite may undergo further biotransformation into a glucuronide conjugate (m/z: 466), which was identified exclusively in rat urine (Figure 1).

Pfizer, on page 21 of its Technical Report 043725 (2020), asserts that the primary metabolite resulting from the de-esterification of ALC-0315 is *6-hexyldecanoic acid* (*m/z*: 255). This finding is further substantiated in Section 3, "Materials and Methods" (page 4), which specifies that the "6-hexyldecanoic acid standard" — the reference substance utilized for instrument calibration, the validation of the analytical method, and the quantification of the hypothetical analyte chemically designated as 6-hexyldecanoic acid — was procured from Millipore-Sigma, St. Louis, Missouri (Figure 2).


PF-07302048 Biotransformation
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ALC-0315

3. MATERIALS AND METHODS

6-HEXYLDECANOIC ACID

3.1. Materials

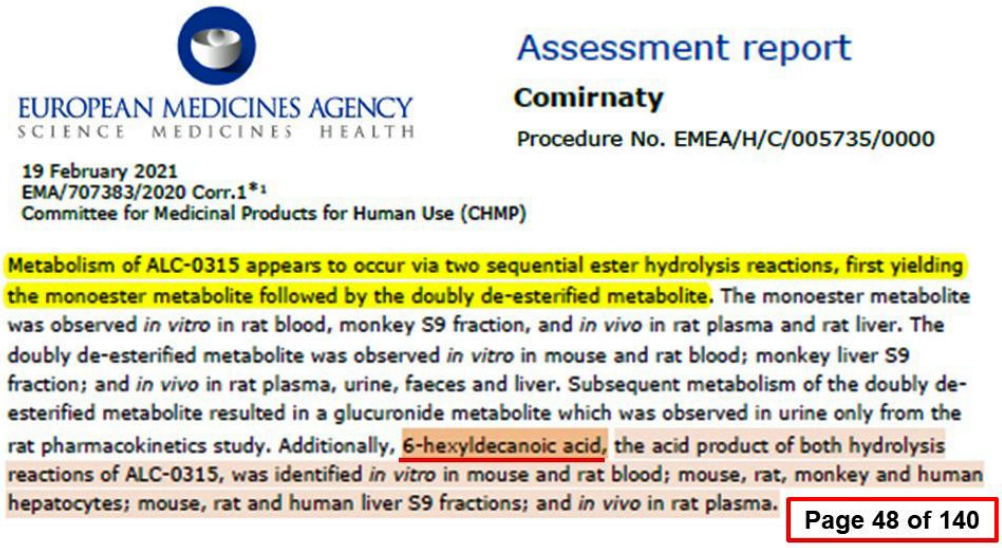
ALC-0159 (2-[(polyethylene glycol]-2000]-*N*,*N*-ditetradecylacetamide, Lot# GALC0159-10), ALC-0315 ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl) bis(2-hexyldecanoate), Lot# GALC0315-11), and Carboxy-MPEG2 (methoxypolyethylene glycol 2000 acetic acid, Lot# 792354-01-011) were obtained from Avanti Polar Lipids, Inc. NAD+, reduced NADH, reduced NADPH, NADP+, alamethicin, adipic acid, diethylene glycol, triethylene glycol, tetraethylene glycol, myristic acid, tetradecylamine, 6-hexyldecanoic acid, 4-aminobutyric acid, and 6-aminohexanoic acid were obtained from Millipore-Sigma (St. Louis, MO). *N*,*N*-Ditetradecylamine was obtained from Ambeed (Arlington Heights, IL). All other reagents were the highest grade commercially available.

Figure 2. Section "Materials and Methods" (from p. 4 Pfizer Report PF-07302048_05Aug20_043725).

The formation and mass spectrometric identification of "6-hexyldecanoic acid" are likewise endorsed by the EMA in its *Comirnaty Assessment Report* dated February 19, 2021, which states on page 48:

> ...*6-hexyldecanoic acid*, the acid product of both hydrolysis reactions of ALC-0315, was identified *in vitro* in mouse and rat blood; mouse, rat, monkey and human hepatocytes; mouse, rat and human liver S9 fractions; and *in vivo* in rat plasma (EMA, 2021; Figure 3).

The documentation produced by Pfizer and subsequently accepted by regulatory authorities appears demonstrably misleading and lacks a rigorous scientific foundation. According to the established principles of organic chemistry and expert consensus, the double de-esterification of the ALC-0315 molecule leads exclusively to the formation of *2-hexyldecanoic acid*, a result dictated by its intrinsic chemical architecture. Additionally, the "6-hexyldecanoic" isomer cited by the manufacturer is neither commercially available nor recognized as a standard for mass spectrometry in established analytical protocols. Consequently, the assertion regarding the use of such a substance as a reference standard


EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

Assessment report

Comirnaty

Procedure No. EMEA/H/C/005735/0000

19 February 2021
EMA/707383/2020 Corr.1*1
Committee for Medicinal Products for Human Use (CHMP)

Metabolism of ALC-0315 appears to occur via two sequential ester hydrolysis reactions, first yielding the monoester metabolite followed by the doubly de-esterified metabolite. The monoester metabolite was observed *in vitro* in rat blood, monkey S9 fraction, and *in vivo* in rat plasma and rat liver. The doubly de-esterified metabolite was observed *in vitro* in mouse and rat blood; monkey liver S9 fraction; and *in vivo* in rat plasma, urine, faeces and liver. Subsequent metabolism of the doubly de-esterified metabolite resulted in a glucuronide metabolite which was observed in urine only from the rat pharmacokinetics study. Additionally, 6-hexyldecanoic acid, the acid product of both hydrolysis reactions of ALC-0315, was identified *in vitro* in mouse and rat blood; mouse, rat, monkey and human hepatocytes; mouse, rat and human liver S9 fractions; and *in vivo* in rat plasma.

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Figure 3. Description of metabolic biotransformation pathway of ALC-0315 according to EMA (Assessment Report Comirnaty, 19 February 2021, Committee for Medicinal Products for Human Use (CHMP). Procedure No. EMEA/H/C/005735/0000).

fails to meet the requisite criteria for scientific and documentary accuracy. The IUPAC nomenclature of the parent molecule itself [(4-hydroxybutyl)azanediyl]di(hexane-6,1-diyl) bis(*2-hexyldecanoate*) — unequivocally identifies the presence of the *2-hexyldecanoic* moiety. This confirms that the declared standard does not correspond to the actual catabolite generated through the molecule's metabolic hydrolysis. It is therefore a matter of profound concern to observe that Pfizer, within official proceedings and throughout documentation essential for securing CMA, claimed to have utilized a reference standard that lacks any basis in the fundamental tenets of organic chemistry. Such a claim — formally reported in a pre-clinical animal study presumably conducted using high-precision analytical techniques — not only disregards basic principles of transparency but fundamentally compromises the reliability of the evidence presented to regulatory bodies. This circumstance can neither be overlooked nor minimized, as it undermines the integrity of the scientific evaluation process and, by extension, the protection of public health. A fundamental question thus arises: does this represent a mere documentary inaccuracy or a nomenclatural error, or must one hypothesize a deliberate omission of pertinent data intended to conceal essential information regarding the chemical-physical safety and toxicological profile of the product submitted to the EMA and FDA? This inquiry is entirely legitimate, as it concerns not only formal regulatory compliance but the safety of billions of individuals. The possibility of willful misconduct (i.e., "*any wrongful act, willingly and knowingly committed, with the intent to cause harmful effects*") is further supported by the fact that both Pfizer and the EMA were fully aware of the presence of 2-hexyldecanoic acid, rather than the 6-hexyldecanoic isomer. This information had already been available since 2017 in patents describing the manufacture of ionizable lipids such as ALC-0315, which provide a detailed account of the synthetic pathway of the compound. These documents clearly indicate that 2-hexyldecanoic acid is one of the primary starting reagents used in the synthesis of ALC-0315 (Boldyrev et al., 2022; Saadati et al., 2022). It is a fundamental tenet of organic chemistry that the hydrolysis of an ester yields the precursor acid and alcohol. If ALC-0315 is synthesized from 2-hexyldecanoic acid, its primary catabolite *must* be 2-hexyldecanoic acid.

The discrepancy becomes even more glaring when comparing the aforementioned Pfizer Report 043725 with the 2021 TGA Australia *Nonclinical Evaluation Report*. On page 11, under the "Novel Excipients" section, the TGA report correctly identifies 2-hexyldecanoic acid as *the* concerned catabolite (Figure 4).

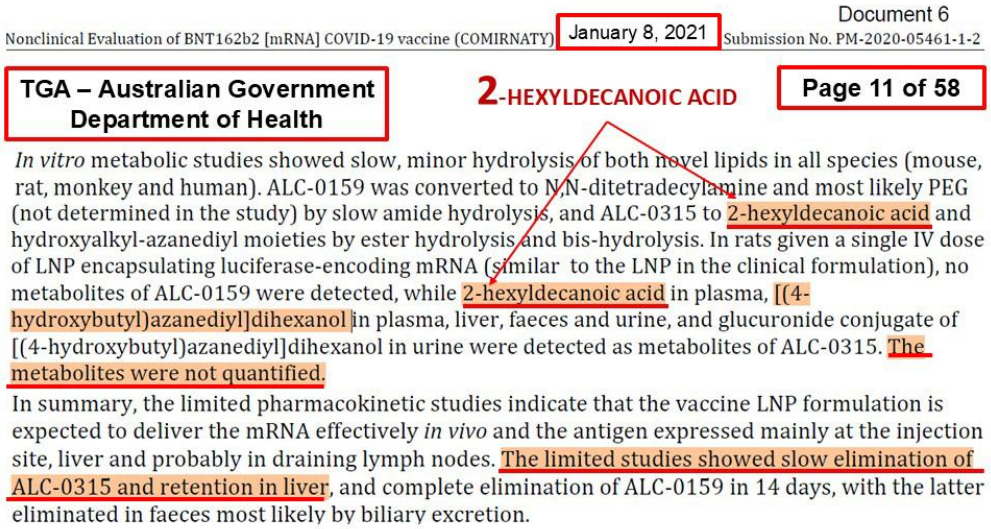

Figure 4. Excerpt from the Australian TGA Nonclinical Evaluation Report BNT162b2 [mRNA] COVID-19 vaccine (COMIRNATY), Document 6, dated January 8, 2021.

In short, Pfizer's 2020 dossier (submitted and endorsed by EMA) *PF-07302048_05Aug20_043725,* (August 5, 2020) describes metabolic-clearance assays calibrated with a purported "6-hexyldecanoic acid" standard — an isomer that does not appear to be traceable as a commercially available reference material — whereas the Australian TGA *Nonclinical Evaluation Report BNT162b2 [mRNA] COVID-19 vaccine (COMIRNATY™), Submission No: PM-2020-05461-1-2*, *Sponsor: Pfizer Australia Pty Ltd* (January 8, 2021) identifies *2-hexyldecanoic acid* as the metabolite produced by ALC-0315 hydrolysis. This documentary discontinuity is difficult to reconcile with a simple clerical mistake: it points instead to either severe negligence in the construction and review of the dossier or to an intentional reframing of the metabolic evidence. In either case, the consequence is the same: the transparency and verifiability of the data submitted to regulators — prerequisites for a sound benefit/risk assessment — are materially compromised.

The use — or claimed use — of a non-verifiable reference standard in mass spectrometry constitutes a serious breach of good laboratory practice (GLP), because it undermines the integrity of identification and quantification based on mass-to-charge ratio (m/z) and retention time (t_R). In practical terms, without a traceable and appropriate standard, neither method validation nor analyte assignment can be considered robust and reliable. Accordingly, Tables 8.5, 8.6, 8.7, and 8.8 of Pfizer Report 043725 (2020) — which report m/z and retention-time values for the "phantom" metabolite "6-hexyldecanoic acid" (m/z 255.2330) — cannot be regarded as scientifically substantiated and should be treated as unreliable for both procedural and regulatory evaluation.

It must further be noted, with profound concern, that the only authentic catabolite generated by ALC-0315 hydrolysis — 2-hexyldecanoic acid — was systematically obscured in the documentation

submitted by Pfizer and subsequently endorsed by EMA, despite being correctly identified by the Australian TGA. This substance (CAS No. 25354-97-6) is classified in the ECHA Chemicals Database under the Globally Harmonized System (GHS) as a hazardous substance, i.e. *Aquatic Chronic 1* (H410: *Very toxic to aquatic life with long-lasting effects*), as well as *Skin Irrit. 2* (H315: *Causes skin irritation*) and *Skin Sens. 1B* (H317: *May cause an allergic skin reaction*; Figure 5).

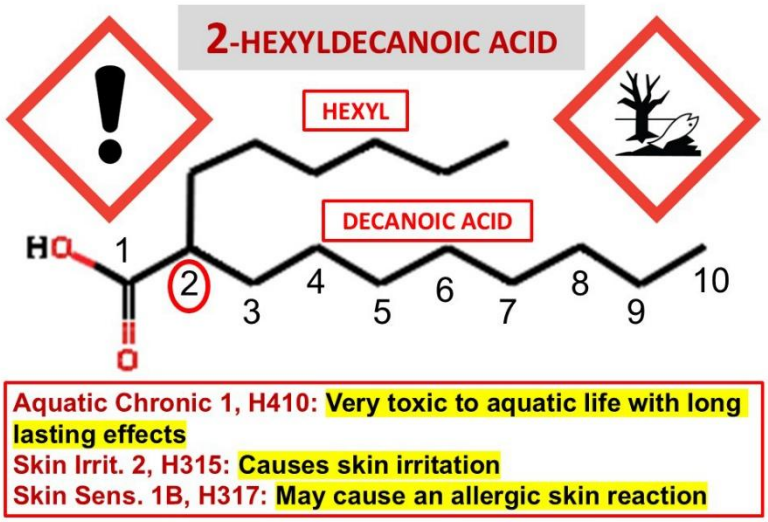

Figure 5. Toxicological classification of 2 hexyldecanoic acid according to Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008.

It is imperative to emphasize that *Aquatic Chronic 1* denotes chronic aquatic toxicity at exceedingly low concentrations — a designation reserved for substances of the highest environmental concern. Such compounds are characterized by marked persistence and limited susceptibility to biodegradation and, in biological systems, to enzymatic processing. Accordingly, this classification carries substantial regulatory implications, including stringent containment obligations and, where relevant thresholds are met, special requirements for facilities storing specified quantities. For context, substances falling under H410 include highly potent pesticides, as well as certain forms of lead and copper alloys (Regulation (EC) No 1272/2008).

In practical terms, 2-hexyldecanoic acid — the metabolite generated by the intracellular hydrolysis of the ionizable cationic lipid ALC-0315 following transfection — constitutes a substance of significant hazard (where 'hazard', according to Directive 2012/18/EU, means *the intrinsic property of a dangerous substance or physical situation, with a potential for creating damage to human health or the environment*), owing to its resistance to metabolic degradation and its consequent propensity for bioaccumulation. Whether these elements were overlooked or suppressed, they warrant serious scientific concern. In this context, it is legitimate to ask whether Pfizer, within its spectrometric, metabolic, and toxicokinetic dossiers, presented "6-hexyldecanoic acid" — a putatively less hazardous and non-verifiable entity — as a "masked metabolite" in order to sidestep the established toxicity profile of the authentic catabolite, the hazardous 2-hexyldecanoic acid.

Such an approach could serve either to construct an apparently acceptable safety narrative or, alternatively, to deflect attention from a persistent and harmful metabolic inhibitor. This interpretation

is reinforced by a clear documentary inconsistency: in 2020, Pfizer described metabolic-clearance assays calibrated with an inert "6-hexyl" standard (i.e., within the submissions preceding the granting of the Conditional Marketing Authorization [CMA]), whereas the Australian Therapeutic Goods Administration (TGA) *Nonclinical Evaluation Report* dated 8 January 2021 — issued *after* the CMA — explicitly identifies *2-hexyldecanoic acid* as the metabolite. The chronology, together with the shift in nomenclature, suggests that the true catabolite was known yet not consistently represented in the primary regulatory dossier.

Current evidence supports the conclusion that *2-hexyldecanoic acid* acts as a potent enzymatic inhibitor — an attribute that, if transparently assessed, would likely have represented a material barrier to approval by EMA Committee for Medicinal Products for Human Use (CHMP). The mechanistic basis is structural: the C2 (α) hexyl branch introduces steric constraints that are not present in the purported C6-branched "phantom" isomer (Figure 6).

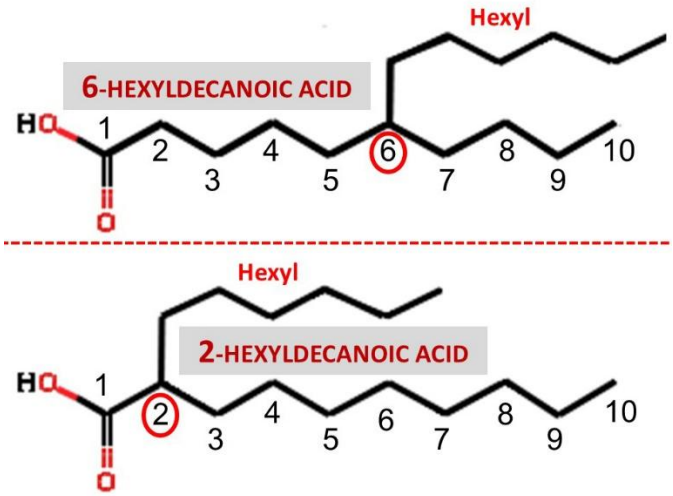

Figure 6. Structural isomers of hexyldecanoic acid and their distinct steric hindrance profiles.

Pfizer reports the use of 6-hexyldecanoic acid as a reference standard, i.e., a C6-branched fatty acid that is readily processed through mitochondrial β-oxidation. By contrast, the true catabolite, *2-hexyldecanoic acid,* bears an α-branch at C2, placing a hexyl substituent adjacent to the carboxyl function. This specific molecular architecture generates marked steric hindrance that impairs substrate accommodation within the active site of acyl-CoA dehydrogenase, thereby inhibiting catalysis (Figure 7) and promoting a persistent blockade of mitochondrial β-oxidation (Fromenty & Pessayre, 1995; Schulz, 2002; Prakash, 2018). It is precisely these structural characteristics that reduce its biodegradability in the aquatic environment, as represented by its H410 classification. In regulatory terms, H410 entails persistence and potential bioaccumulation at low concentrations. Unlike the alleged 6-hexyldecanoic isomer — which lacks a traceable CAS number — the actual acid catabolite of ALC-0315, 2-hexyldecanoic acid (CAS No. 25354-97-6) has a documented hazard profile, namely *Aquatic Chronic 1*, high bioaccumulation potential (*logP* > 6), poor aqueous solubility, and limited biodegradability.

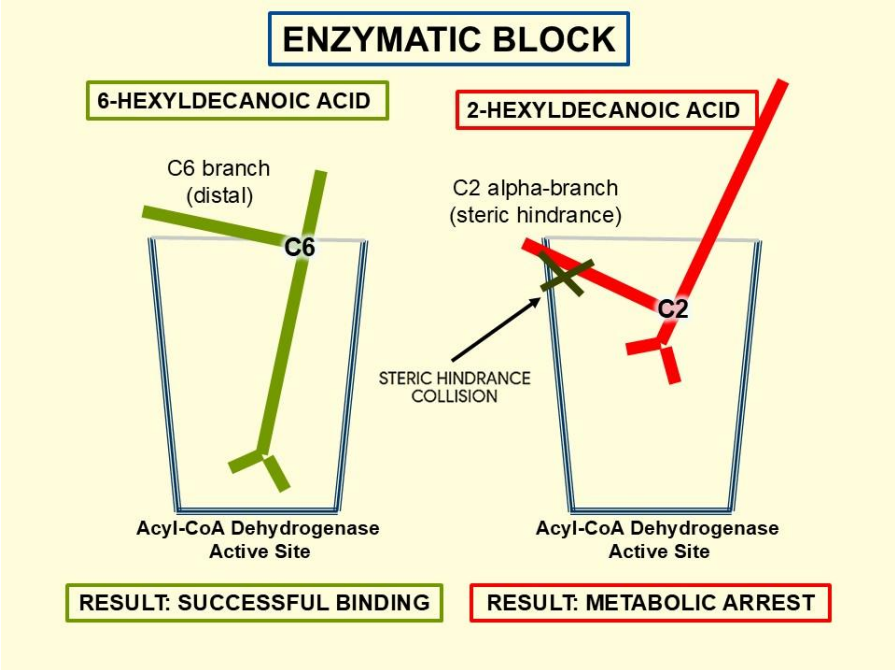

Figure 7. The C2 (α)-branched 2-hexyldecanoic acid induces steric hindrance that limits docking in acyl-CoA dehydrogenase, inhibiting mitochondrial β-oxidation, unlike the C6-branched 6-hexyldecanoic acid.

Inhibition of β-oxidation by a branched fatty acid can rapidly compromise cellular bioenergetics by limiting ATP production and perturbing NAD^+ recycling. The resulting decline in reducing equivalents (NADH and $FADH_2$) impairs the electron transport chain and oxidative phosphorylation, thereby promoting reactive oxygen species (ROS) generation (Hou et al., 2021). ATP depletion in dysfunctional mitochondria may, in turn, weaken nuclear integrity by depriving the cell of the energy required for repair of DNA damage induced by oxidative stress. In addition, ROS can be amplified by nanomaterials and cationic ionizable lipids that resist cytosolic degradation, further increasing toxicological burden (Yun et al., 2016; Yu et al., 2020; Segalla, 2023b).

In summary, the pharmacokinetic and metabolic dossiers for Pfizer's ALC-0315 raise substantial ethical and deontological concerns and indicate a manifest breach of Good Laboratory Practice (GLP), resulting in:

1. Failure to identify and assess the hazardous metabolite, *2-hexyldecanoic acid*, classified as *Aquatic Chronic 1* (H410: *Very toxic to aquatic life with long-lasting effects*) and known to act as a mitochondrial inhibitor.
2. Submission of analytically questionable method validation, which undermines the integrity, transparency, and reproducibility of the data transmitted to regulatory authorities.
3. Avoidance of a comparative toxicological assessment of the α- (C2) branching in 2-hexyldecanoic acid, which is directly correlated with enzymatic inhibition when compared to isomers possessing distal branching.
4. Scientific invalidity of the spectrometric tables reported in Pfizer Report 43725, with the potential implication of procedural fraud and a severe breach of GLP protocols.

5. Evidence that the EMA endorsed the "spurious" molecule 6-hexyldecanoic acid (EMA, 2021; Figure 3) without rigorous verification of traceability and analytical compliance, leading to the publication of an official Assessment Report that validates a chemical state which is implausible according to the fundamental tenets of ester hydrolysis.

3.1. THE TRIALKANOLAMINE TRAP

The double de-esterification of ALC-0315 (Figure 1) yields, in addition to two moles of 2-hexyldecanoic acid, one mole of a hydroxylated tertiary amine. This aminic catabolite comprises a 4-hydroxybutyl chain (with the hydroxyl group at the δ position, 4 carbon atoms away from the nitrogen atom) and two 6-hydroxyhexyl chains (with the hydroxyl groups at the ζ position, 6 carbon atoms away from the nitrogen atom). Its structural formula is $N(C_4H_8OH)(C_6H_{12}OH)_2$, with the chemical designation *[(4-hydroxybutyl) azanediyl] dihexanol* and a molecular weight of 289.46 g/mol (Figure 8).

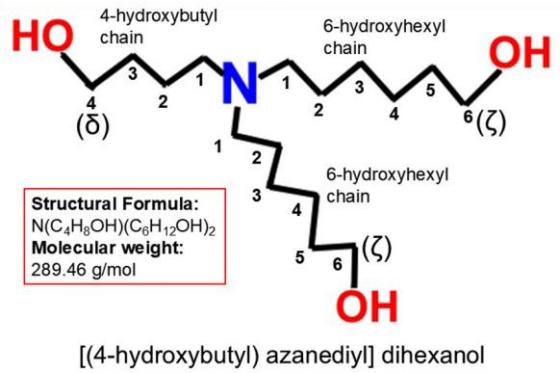

Figure 8. Chemical structure of the trialkanolamine catabolite generated by ALC-0315 hydrolysis ([(4-hydroxybutyl)azanediyl]dihexanol; MW 289.46 g/mol).

Regarding this amine metabolite and its corresponding mass-spectrometric assessment, it is difficult not to note a serious inconsistency in Report 043725 (2020). Pfizer reports both the mass-to-charge ratio (m/z 290.2690) and the retention time (t_R 8.1 min) under "Bis-hydrolysis (amine)", and even provides an accurate structural depiction of the trialkanolamine (Figure 1); yet the compound is conspicuously missing from the list of certified reference materials (standards) used for UHPLC-MS/MS validation (Figure 2). Presenting metabolic-clearance data for a metabolite in the absence of any traceable standard is, at best, a glaring methodological lapse — and one that should never have been allowed to pass unchallenged in a regulatory dossier.

This omission nullifies any credible claim of identification and, even more so, of quantification: without a certified standard, the assignment of peak identity and the calculation of tissue levels lack the minimum analytical guarantees required in regulatory toxicology. As a result, the reported concentrations rest on assumptions rather than validated measurements, calling into question the reliability of the methodology employed in a pre-clinical phase that should, by definition, provide the evidentiary foundation for human trials and, ultimately, for marketing authorization. In continuity with the already troubling "6-hexyldecanoic acid" episode, the same pattern reappears here: essential mass-spectrometry requirements are sidestepped, and GLP compliance is effectively reduced to a formal label. When the reference standard is missing, method validation is not merely weakened — it is void,

and the resulting dataset cannot be treated as scientifically dependable for either toxicological assessment or regulatory decision-making.

We therefore turn to what this omission effectively keeps out of view: the mechanistic and regulatory implications attached to this second "phantom" metabolite, and the reasons why the underlying analytical evidence appears to have been treated with such unwarranted permissiveness.

The pKa value (acid dissociation constant) of the trialkanolamine derived from the hydrolysis of ALC-0315 can be reliably estimated using the SPARC (*SPARC Performs Automated Reasoning in Chemistry*) computational method (Hilal et al., 2003). This advanced system leverages the molecular architecture of organic compounds to accurately predict various physicochemical properties, including the pKa of acids and bases. SPARC is based on mechanistic perturbation models that incorporate electronic, solvation, and hydrogen-bonding effects, providing a detailed and rigorous assessment of molecular characteristics. Such computational tools are indispensable for obtaining reliable and reproducible data, particularly when the target molecule is unavailable as a commercial standard and is absent from major international chemical databases.

The calculated pKa for the trialkanolamine byproduct of ALC-0315 hydrolysis accounts for the inductive attenuation coefficient resulting from the presence of the 4-hydroxybutyl chain (with the -OH group placed 4 carbon atoms away from the nitrogen atom) and the two 6-hydroxyhexyl chains (with the -OH group placed 6 carbon atoms away from the nitrogen atom; Figure 8). These inductive effects, which modulate the dissociation capacity of the molecule, are essential for a realistic estimation of the chemical behavior of the trialkanolamine. Based on these considerations, the pKa of this compound is estimated to range between 9.1 and 9.6. This value determines its predominant ionic state across various cellular compartments and its ability to permeate biological membranes.

To characterize the distribution of the trialkanolamine across cellular compartments, the Henderson–Hasselbalch equation is applied using the pKa values obtained with the SPARC computational framework. This relationship quantifies the equilibrium ratio between the protonated cationic form (BH^+) and the neutral base (B) as a function of environmental pH:

$$[BH^+]/[B] = 10^{(pKa - pH)}$$

Accordingly, the equation provides a quantitative estimate of the relative abundance of the two species, enabling a rigorous description of the compound's pH-dependent *speciation* (i.e. its distribution among the two above defined chemical species) and, by extension, its compartmentalization potential.
At cytosolic pH (≈ 7.2), the calculated speciation indicates that BH^+ represents 98.76% (pKa = 9.1) to 99.60% (pKa = 9.6) of the trialkanolamine molecular population. The neutral fraction is therefore minimal; nevertheless, it is mechanistically decisive, because only the uncharged species can diffuse across lipid bilayers and mediate inter-compartmental translocation.

By contrast, in the lysosomal lumen (pH ≈ 4.5), where proton activity is markedly higher, protonation is essentially quantitative: BH^+ reaches 99.99% (pKa = 9.1) and approaches 100% (pKa = 9.6). Under these conditions, the molecule acquires a quasi-permanent positive charge that prevents back-diffusion through the lysosomal membrane, thereby establishing "ion trapping" (lysosomotropism), as we will see in the next section.

3.2. THE "ONE-WAY GATE" EFFECT

In summary, at cytosolic pH (≈ 7.2), an infinitesimal fraction (≈ 0.40%–1.25%) of the trialkanolamine exists in its neutral form, enabling it to permeate phospholipid bilayers and enter the lysosomes. Upon entry into the lysosomal compartment, where the pH is ≈ 4.5, the molecule encounters a proton H^+ concentration nearly 1,000-fold higher. Under these acidic conditions, protonation becomes quasi-absolute (≈100%). In its fully ionized state (BH^+), the trialkanolamine acquires a permanent positive charge that precludes back-diffusion across the lysosomal membrane — which is impermeable to ions — resulting in sequestration within the lysosomal lumen (Figure 9).

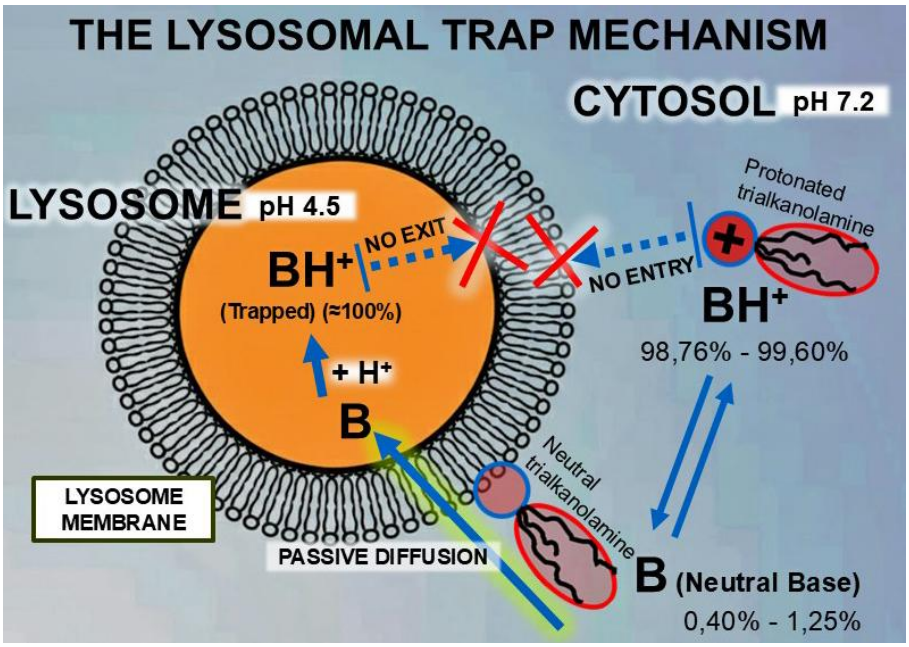

Figure 9. Lysosomal "ion trapping" of the trialkanolamine: pH-dependent protonation drives sequestration within the lysosomal lumen and prevents back-diffusion.

Outcome: The sequestered trialkanolamine binds electrostatically to negatively charged phospholipids of the lysosome membrane, forming indigestible lipid-protein complexes. The presence of these complexes inhibits lysosomal enzymes, specifically phospholipases, thereby arresting autophagy — the essential cellular process responsible for the degradation of damaged organelles, such as mitochondria compromised by 2-hexyldecanoic acid. This results in *iatrogenic phospholipidosis* (Anderson and Borlak, 2006), characterized by the pathological accumulation of lipid lamellae within the organelles and the total blockade of autophagic flux (Figure 10).

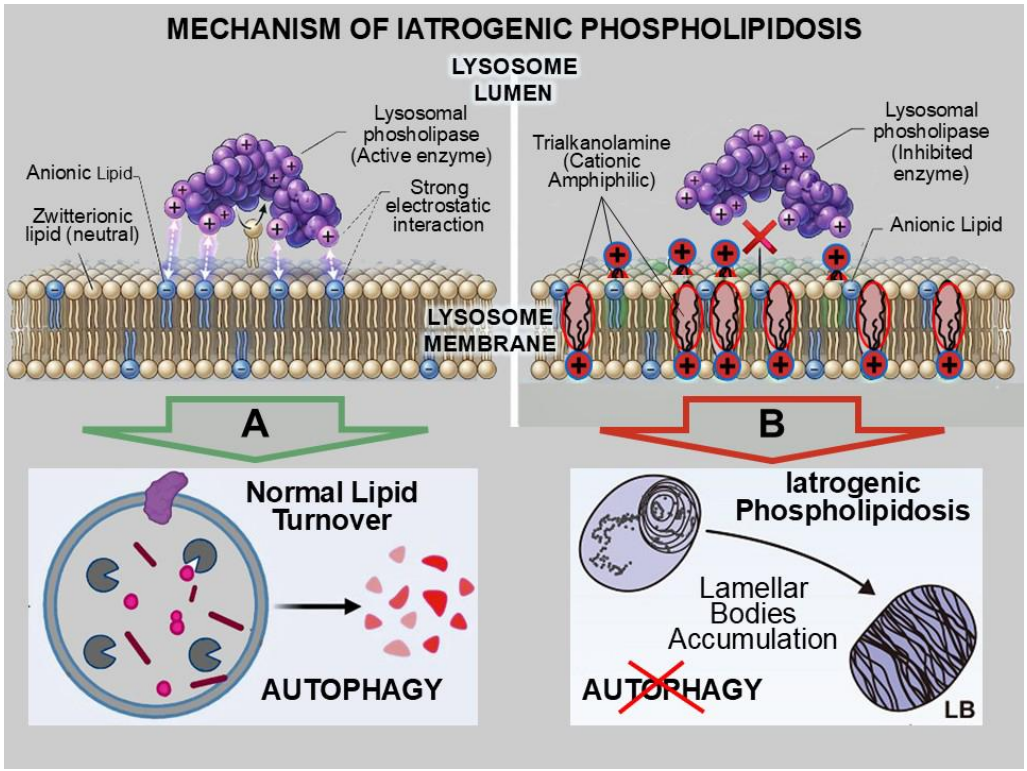

Figure 10. A) The autophagy-lysosome pathway: a fundamental intracellular catabolic system that efficiently degrades inner membranes. B) Iatrogenic phospholipidosis: accumulation of indigestible lipid–protein complexes inhibits lysosomal phospholipases, blocking autophagic flux.

The lysosome functions not merely as a "metabolic waste receptacle" but as the regulatory core of the autophagic system. When lysosomes become saturated with the metabolic cargo of ALC-0315 catabolites, the entire autophagic cascade reaches a state of functional congestion, leading to systemic arrest. The cell loses its capacity for homeostatic renewal, undergoing a form of "physical suffocation": the accumulation of biochemical debris congests the cytoplasm, disrupting intracellular trafficking and precipitating premature senescence or cell death via necrosis/apoptosis.

These dysfunctional cells secrete pro-inflammatory cytokines, fostering a chronic inflammatory microenvironment that may exacerbate pre-existing pathologies or trigger novel autoimmune processes, as hypothesized in studies regarding the adjuvant activity and toxicological risks of lipid nanoparticles (LNPs). These findings suggest that the resulting damage is not an acute, transient event, but rather a cumulative process (Segalla, 2023b; Segalla, 2024).

By failing to evaluate the actual clearance of these metabolites using valid reference standards, Pfizer significantly underestimated the risk of autophagic paralysis, condemning cells to a state of metabolic "suffocation" that underpins the chronic toxicity and long-term risks associated with mRNA-conjugated lipid nanomaterials.

3.3. THE "DETERGENT" MECHANISM: NUCLEAR MEMBRANE DESTABILIZATION AND NUCLEAR INFILTRATION

The conversion of the ALC-0315 lipid into its trialkanolamine catabolite represents more than mere degradation; it is a profound chemical metamorphosis. From an apparently inert lipid structure emerges an amphiphilic tertiary amine characterized by high cytotoxicity.
The octanol-water partition coefficient (logP) serves as a primary indicator of a neutral molecule's lipophilicity. ALC-0315, characterized by four long hydrocarbon chains, is virtually insoluble in water — a "super-lipid" destined to remain sequestered within the phospholipid bilayer. According to the *XLogP3* predictive model (Cheng et al., 2007), the logP of ALC-0315 exceeds 12. However, enzymatic hydrolysis (de-esterification) removes the two 2-hexyldecanoic acid chains, leaving only the short, hydroxyl-terminated (-OH) chains characteristic of trialkanolamines. This results in a drastic decline in lipophilicity: the estimated logP for the generated trialkanolamine falls between 1.5 and 2.5.
For ionizable basic molecules, the distribution coefficient (logD) provides a more precise measure of hydrophilicity at a specific pH, as defined by the formula:

$$\mathbf{logD = logP - log(1 + 10^{(pKa-pH)})}$$

With a log P of ≈ 2, a pKa between 9.1 and 9.6, and a cytosolic pH of 7.2, the logD of the trialkanolamine is near zero or negative (+0.09 to –0.4). Generally, a high logD value indicates greater lipophilicity, while a low or negative value reflects prominent hydrophilicity; a logD near zero identifies an amphiphilic molecule that is easily dispersible within the aqueous cytosolic matrix. The transition from the logP ≈ 12 of ALC-0315 to a logP ≈ 2 and logD ≈ 0 for its amine metabolite transforms the poorly soluble parent lipid into a highly water-dispersible cationic amine surfactant capable of solubilizing and destabilizing cellular lipids. Due to its low logD and net positive charge, the trialkanolamine behaves as an aggressive cationic detergent, analogous to cetyltrimethylammonium bromide (CTAB), which is utilized in laboratory settings for its capacity to disrupt lipid membranes. While the ALC-0315 molecule remains largely confined within the phospholipid membrane due to its massive lipophilic architecture, the protonated and hydrodispersible trialkanolamine migrates readily through the cytosol. Upon reaching the nuclear envelope, its amphiphilic nature facilitates its insertion between phospholipids, where it interacts with negatively charged phospholipid heads via its own positive charge. This process destabilizes the nuclear envelope — a phenomenon known as "lipid stripping" (Sudbrack et al., 2011) — and once the membrane is compromised, the cationic trialkanolamine can interact directly with the negatively charged portions of DNA, potentially obstructing transcription and repair mechanisms (Figure 11).

The precipitous decline in logP from a value >12 to approximately 2 marks a critical phase: the lipid is transformed from a purely structural component into a hydrophilic cationic compound possessing surfactant properties. This transition enables the destabilization of phospholipid bilayers, entailing significant cytotoxic and genotoxic ramifications. The trialkanolamine — characterized by a pKa ≈ 9.6, logP ≈ 2.0, and logD ≈ 0 — was not rigorously characterized by Pfizer, resulting in an underestimation of the "lipid stripping" effects exerted on intracellular membranes, including the nuclear envelope. Such effects may compromise genomic integrity and facilitate oncogenic pathways associated with nuclear membrane instability. As we have already seen, the first catabolite of ALC-

0315, 2-hexyldecanoic acid, inhibits β- oxidation and the mitochondrial respiratory chain, precipitating a depletion of cellular ATP. Deprived of essential metabolic energy, the cell is unable to repair DNA

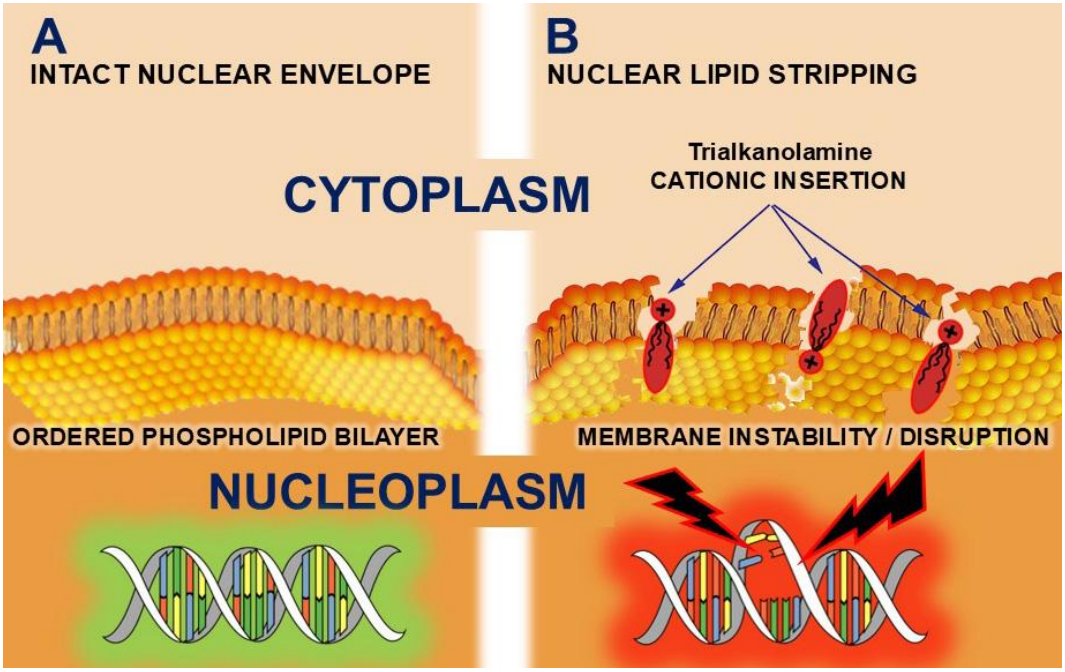

Figure 11. A) Ordered phospholipid bilayer of the intact nuclear membrane B) Detergent-like behavior of the trialkanolamine disrupts the nuclear envelope ("lipid stripping"), enabling nuclear infiltration and direct interactions with DNA.

lesions induced by oxidative stress and lipid stripping. A deleterious cycle is thus established: the trialkanolamine compromises nuclear structural stability, while 2-hexyldecanoic acid obstructs the repair of biochemical damage. Persistent trialkanolamine-phospholipid interactions may induce micro-lesions in the nuclear membrane, facilitating the influx of cytoplasmic enzymes or the efflux of genetic material. These phenomena are intrinsically linked to cellular senescence and neoplastic transformation — findings that stand in direct contradiction to regulatory dossiers regarding the presumed "inertia" of the lipid excipients:

"Genotoxicity or carcinogenicity studies were not performed. The components of the vaccine formulation are lipids and RNA that are not expected to have genotoxic potential." (EMA/707383, 2021).

The omission of the trialkanolamine analytical standard in mass spectrometric evaluations (Report 043725, 2020) precluded the experimental determination of its logP and pKa values. In the absence of verifiable data, Pfizer issued "clearance" claims based on neglected or omitted standards, or even fabricated data, such as the "phantom 6-hexyldecanoic acid". This approach obfuscates the actual catabolic fate of ALC-0315 and contravenes the fundamental principles of scientific transparency and rigor. After getting rid of the LNP disassembled by the endosomes, ALC-0315 undergoes biotransformation into an organic acid capable of inhibiting β-oxidation and a cationic tertiary amine with aggressive detergent properties. The latter is distinguished by high mobility (low logD), cytotoxicity (being almost entirely protonated at pH 7.2), and potent interactions with membrane

phospholipids, in a similar way to what happens for strong cationic surfactants. The intracellular accumulation of recalcitrant catabolites fosters a state of chronic inflammation. Coupled with nuclear membrane destabilization, reactive oxygen species (ROS) generation, and ATP deficits, this environment promotes genomic instability — the susceptibility of genetic material to structural and functional alterations. This condition is a primary precursor to oncogenic development and neoplastic transformation. In the case of ALC-0315 metabolites, the risk is amplified by both the structural membrane damage induced by the amine catabolite's detergent properties and the bioenergetic crisis triggered by the acid catabolite's metabolic blockade. Despite these risks, Pfizer neglected to conduct comprehensive investigations into the genomic instability induced by ALC-0315 hydrolysis, notwithstanding the clear cytotoxic indicators associated with the high intrinsic pKa of such ionizable cationic lipid, which enhances its capacity to disrupt and destabilize cellular architectures (Yun et al. 2016; Yu et al. 2020; Patel et al. 2021; Ndeupen et al. 2021; Palmer et al. 2022; Segalla, 2023a; Segalla, 2023b).

3.4. THE FAILURE OF GLUCURONIDATION: A REGULATORY GAP IN METABOLIC CLEARANCE EVIDENCE

In Pfizer Report 043725 (2020), the final metabolite discussed — once again without a pre-characterized spectrometric reference standard — is the amine-glucuronide (m/z 466; t_R 7.9 min). This species is described as a conjugate bearing a single glucuronic acid moiety, reportedly formed at one hydroxyl group. Glucuronic acid is the canonical substrate of the *UDP-glucuronosyltransferase* (UGT) superfamily, a central Phase II detoxification system (Mohsin et al. 2024) primarily expressed in liver — and, to a lesser extent, in intestine and kidney — whose catalytic function is to increase aqueous solubility and promote renal or biliary elimination of endogenous compounds (e.g., bilirubin, hormones) and xenobiotics (drugs, toxins; Rowland et al., 2013). However, as detailed above, the trialkanolamine generated by ALC-0315 hydrolysis is predicted to undergo massive cationic sequestration in lysosomes. This point is decisive, because UGT enzymes are predominantly localized to the lumen of the endoplasmic reticulum (ER). A protonated species (BH^+) physically trapped in an acidic lysosomal compartment has sharply reduced cytosolic availability and, consequently, limited access to ER-resident UGTs. The reported detection of mono-glucuronides therefore implies that glucuronidation — if occurring — can only involve the infinitesimal free-base fraction (0.4%–1.25%) that transiently escapes lysosomal trapping, effectively turning "clearance" into a stochastic process rather than a structured and efficient elimination pathway.

Stated differently, the dossier points to a highly partial — if not practically negligible — glucuronidation, limited to a single hydroxyl among the three available on the trialkanolamine scaffold. This mechanistic rationale is consistent with cellular compartmentalization: with ≈ 99.6% of the trialkanolamine protonated and retained in lysosomes, the substrate is effectively segregated from ER-localized UGTs, rendering conjugation intrinsically inefficient. Yet even this limited claim remains analytically fragile, because the absence of certified standards for both the parent trialkanolamine and the trialkanolamine-monoglucuronide is not a minor omission: it is an *impermissible* gap for a regulatory-grade UHPLC-MS/MS dataset.

The fact that Pfizer reports only trace levels of monoglucuronide in rat urine is, in this context, more consistent with "background noise" generated by the minimal unprotonated fraction *prior* to sequestration than with a meaningful biotransformation route. The bulk of the trialkanolamine remains

confined within the lysosomal acidic milieu, where the BH^+ form is not a viable substrate for microsomal enzymes. Moreover, the low logD (≈ 0) denotes an intrinsically hydrodispersible species with limited affinity for UGTs, which typically process more lipophilic substrates. Taken together with the complete absence of poly-glucuronidated derivatives in Pfizer Report 043725 (2020), these elements indicate that the metabolic Phase II pathway is, in practice, biochemically precluded; the identified mono-glucuronide is best interpreted as a quantitatively irrelevant metabolic artifact.
It is particularly striking — and, from a regulatory perspective, difficult to justify — that in the absence of certified analytical standards for the trialkanolamine and its glucuronide, Pfizer could not rigorously determine the amount of unmetabolized parent material. Presenting the m/z 466 mono-glucuronide as evidence of "metabolic clearance" is therefore not merely scientifically unsubstantiated; it constitutes a serious irregularity in evidentiary terms. Without standards, it is impossible to verify whether this elimination route accounts for a marginal fraction (potentially <0.1% of the administered dose), leaving open — unacceptably, for a dossier supporting human exposure — the prospect of substantial bioaccumulation.

Absent a rigorous mass-balance quantification, the mere qualitative detection of glucuronide traces in rat urine risks functioning as a rhetorical surrogate for elimination, while the core toxicological question remains unresolved: whether, and to what extent, the administered dose persists within tissues. If catabolic elimination is as marginal as the above constraints suggest, then the pharmacokinetic premise shifts from clearance to retention, with predictable consequences — intrinsic cytotoxicity and membrane destabilization — rather than prompt removal. Such an evidentiary posture is incompatible with the transparency expected in regulatory pharmacology and raises serious concerns about long-term toxicological liabilities and the permissiveness of the review pathway that accepted these data (Segalla, 2023a; 2023b).

4. RIBOSOMAL SABOTAGE AND FRAMESHIFTING: POTENTIAL ONCOLOGICAL AND CARDIOLOGICAL CONSEQUENCES

Under oxidative stress driven by ROS, the incomplete degradation of ALC-0315 and its downstream metabolites can culminate in lipid peroxidation and the generation of reactive aldehydes, notably 4-*hydroxynonenal* (4-HNE) and *malondialdehyde* (MDA). These species are not merely biomarkers but reactive effectors capable of forming covalent adducts with proteins and with the vaccinal mRNA, thereby altering macromolecular structure and function and amplifying oxidative-stress pathology (Dalleau et al., 2013; Shoeb and al., 2014; Barrera et al., 2018; Palmer and Bhakdi, 2021; Palmer et al., 2022; Li et al., 2022; Birdsall et al., 2024; Ioannidis et al., 2025).

This molecular damage landscape provides a plausible trigger for *ribosomal stalling*, i.e., the immobilization of translating ribosomes, with a downstream propensity to *frameshifting* (a shift of the reading frame during translation; Willi et al., 2018; Shcherbik et Pestov, 2019). Stalling is not a neutral interruption: it activates proteotoxic stress pathways and consumes resources already constrained by the ALC-0315-driven "bioenergetic vampirism". Mechanistically, the ribosome remains locked onto the mRNA template, impeding physiological protein synthesis and favoring aberrant translational outcomes, including truncated Spike products and — more critically — chimeric peptides lacking homology with the intended wild-type sequence. The accumulation of truncated and misfolded proteins can exceed cellular degradative capacity, producing "proteic debris" that disrupts proteostasis and sustains chronic stress signaling. In parallel, depletion of NAD^+ — a required cofactor for DNA

repair enzymes such as poly(ADP-ribose) polymerase (PARP) — weakens genomic maintenance, thereby establishing a biochemical context permissive to genomic instability and neoplastic transformation (Rajman et al., 2018; Xie et al., 2020).

Within this framework, the cell may become unable to sustain functional protein output and to preserve genomic stability, thereby eroding the regulatory checkpoints that normally restrain unregulated proliferation. The resulting imbalance plausibly channels the system toward two non-exclusive pathological trajectories: (i) aberrant proliferative signaling that favors oncogenesis, and/or (ii) a senescent phenotype sustained by chronic inflammatory signaling. Recent literature has associated these conditions with aggressive oncological manifestations reported following administration of the vaccine preparation (Goldman et al., 2021; Parry et al., 2023; Abue et al., 2025; Marik and Hope, 2025; Kuperwasser and Wafik, 2026; Gentilini et al., 2026;).

mRNA vaccines have also been associated with inflammatory cardiac sequelae, notably myocarditis and pericarditis, with a comparatively higher incidence in younger cohorts. Although clinical evolution is reported as favorable in most cases, severe and, in rare circumstances, fatal outcomes have been documented (Jablonowski and Hooker, 2022; Oster et al., 2022; Santiago and Oller, 2023; Yu et al., 2023; Yasmin et al., 2023; Yonker et al., 2023; Mead et al., 2024a; Mead et al., 2024b; Hulscher et al., 2024). A mechanistic link to ribosomal frameshifting is biologically plausible because the heart is composed largely of post-mitotic cardiomyocytes with limited regenerative capacity; consequently, even transient episodes of aberrant protein synthesis and immune-mediated injury may translate into disproportionate functional impact. In this context, frameshifting should be viewed not simply as a translational irregularity, but as a plausible upstream contributor to cardiac immunopathology: by generating novel peptide sequences, it may create neo-antigens that elicit targeted immune recognition within a largely post-mitotic tissue with limited capacity to replace damaged cells.

Lipid nanoparticles (LNPs), whose systemic stability is enhanced by a low apparent pKa, may persist in circulation long enough to reach cardiomyocytes via the bloodstream. After cellular uptake, LNPs release the mRNA cargo, which is then translated by host ribosomes within the cardiomyocyte cytoplasm. If frameshifting occurs in this context, translation may yield aberrant and chimeric proteins that diverge from the intended primary sequence. Because these products are exogenous to host biology, they can be processed and recognized as non-self through established immunological mechanisms, including:

- *Generation of foreign proteins*: Frameshifting-derived products may display anomalous amino-acid sequences that depart from physiological host proteins, increasing immunogenicity and facilitating their recognition as potentially pathogenic.
- *MHC-I presentation*: Following synthesis, cardiomyocytes can present peptides derived from aberrant proteins on the cell surface via the Major Histocompatibility Complex Class I (MHC-I), enabling immune surveillance of altered intracellular translation products.
- *$CD8^+$ cytotoxic T-cell engagement*: $CD8^+$ T-lymphocytes may interpret these presented neo-antigens as hallmarks of infection, genetic alteration, or neoplastic transformation and initiate cytotoxic clearance of the cardiomyocytes. This immune cascade can culminate in acute myocarditis with potential impairment of organ function.

Furthermore, frameshifting-derived chimeric or truncated proteins may expose surface epitopes — short amino-acid motifs — that, by chance or partial structural homology, resemble epitopes present on endogenous human proteins. Such molecular mimicry can blur the self/non-self boundary, increasing the likelihood of immune cross-targeting and thereby expanding injury from "foreign" products to native cardiac tissues, via mechanisms such as:

- *Cross-reactivity*: If a frameshifting-derived chimeric protein displays sufficient similarity to cardiac α-myosin or to desmosomal structural proteins essential for cardiomyocyte cohesion, antibodies and T-lymphocytes primed against the "pseudo-Spike" may cross-react with native targets, triggering autoimmune myocardial injury.
- *Pericardial inflammation*: Analogously, mimicry involving proteins expressed by pericardial mesothelium may lower the activation threshold for immune targeting of pericardial tissues, facilitating pericarditis as a downstream consequence of an aberrant immune response.

As documented in numerous studies, lipid nanoparticles (LNPs) should not be treated as inert vectors but as platforms with potent pro-inflammatory adjuvant activity (Zaman et al., 2013; Swaminathan et al., 2016a, 2016b; Tizard, 2020; Sahin et al., 2020; Verbeke et al., 2022; Ndeupen, 2021; Alameh et al., 2021; Tahtinen et al., 2022). In a cellular milieu already biased toward inflammation by ALC-0315 residues and toxic catabolites, the concurrent presence of aberrant translation products generated via frameshifting can further lower immunological tolerance thresholds and intensify tissue-level inflammatory signaling.

The NLRP3 inflammasome is a cytoplasmic multiprotein complex of the innate immune system that senses cellular stress and danger signals (Kelley et al., 2019). In cardiac tissue, the combined exposure to LNP-driven inflammatory cues and to chimeric protein products may promote hyperactivation of NLRP3 within resident cardiac macrophages, resulting in increased release of pro-inflammatory interleukins, particularly IL-1β and IL-18, which are mechanistically implicated in myocarditis. Overall, the convergence of (i) LNP adjuvanticity, (ii) the emergence of aberrant translational products, and (iii) immune dysregulation delineates a coherent pathway toward intense and potentially deleterious inflammatory responses in cardiac tissue. In this regard, it is notable that enhanced translational efficiency of vaccinal mRNA — enabled by substitution of uridine with N1-methyl-pseudouridine — has been hypothesized to increase the probability of ribosomal frameshifting (Federico, 2024; Mulroney et al., 2024; Rubio-Casillas et al., 2024). If so, the resulting spectrum of unintended, immunogenic protein products would expand the range of plausible biological effects intrinsic to the mRNA/LNP platform.

5. CONCLUSIONS AND PERSPECTIVES

The conditional marketing authorization (CMA) granted to the Pfizer/BioNTech product appears to rest on a materially inaccurate representation of the metabolic fate of the ionizable lipid ALC-0315. In particular, the regulatory narrative — as reconstructed from the submitted dossiers — relies on the citation of chemical entities that are not demonstrably traceable, in lieu of the authentic catabolites that would be expected on elementary chemical grounds. Such a substitution is not a marginal technicality: it potentially vitiates the evidentiary basis of the assessment and, by extension, undermines the integrity

of the large-scale benefit/risk evaluation. In this context, the remarks attributed to Helmut Sterz, former Chief Toxicologist at Pfizer, are noteworthy: he reportedly considered the preclinical package advanced by Pfizer/BioNTech insufficient to robustly guarantee the safety of trial participants and, subsequently, of the general population. In *Die Impf-Mafia* [The Vaccination Mafia, 2026], he further argues that emergency conditions do not abrogate institutional duties; competent authorities retain the obligation to demand rigorous safety evidence within their pharmacovigilance remit. This reminder is uncomfortable yet unavoidable: when the evidentiary bar is lowered, responsibility cannot be displaced solely onto manufacturers. A structured comparison among Pfizer Report 043725 (*Materials*, p. 4), the Australian TGA *Nonclinical Evaluation Report* (2021; Pharmacokinetics — Novel Excipients, p. 11), and the EMA *Comirnaty* Assessment Report (19 February 2021; Section 2.3.2, p. 48) highlights internal inconsistencies in how the toxicological and metabolic evidence was represented and then propagated into regulatory conclusions, without an adequately confrontational review. On the basis of the mechanistic reconstruction developed in the present work, ALC-0315 should be considered not merely a functional LNP excipient, but a bioactive precursor capable of interfering with core cellular systems through at least three convergent pathways:

- Mitochondrial β-oxidation inhibition, mediated by *2-hexyldecanoic acid* (H410; *Aquatic Chronic 1*).
- Lysosomal homeostasis disruption and lysosomotropic sequestration, driven by the cationic trialkanolamine *[(4-hydroxybutyl)azanediyl]dihexanol*.
- Nuclear-envelope destabilization ("lipid stripping") through detergent-like membrane perturbation.

Overall, the safety profile of ALC-0315, as portrayed in the examined documentation, is challenged here on three tightly linked grounds: (i) the use of inadequately documented analytical standards, (ii) the omission — methodological and interpretative — of critical toxicological features of the amine-derived catabolites, and (iii) the downstream biological consequences discussed above, including ribosomal sabotage. The alleged use of a "6-hexyldecanoic" standard emerges as a central — and difficult to excuse — criticality, because it redirects the assessment toward a theoretical and readily degradable isomer rather than the authentic metabolite, *2-hexyldecanoic acid*, which carries an H410 classification (*Very toxic to aquatic life with long-lasting effects*). In regulatory terms, H410 triggers an obligation to address persistence and bioaccumulation even at low exposure levels; yet the corresponding chronic-risk profile is not shown to have been adequately explored, either by the MAH or by the reviewing authorities. In Report 043725, Pfizer states that the LC-MS/MS methods were validated using "6-hexyldecanoic acid" as reference standard. However, this entity is reported to lack a traceable CAS number, to be absent from major reference databases (e.g., PubChem, NIST), and to be unavailable in commercial catalogues (e.g., Millipore Sigma). If so, the validation claim is not merely weak; it becomes evidentially non-verifiable. As a consequence, the robustness of clearance determinations — including linearity and limits of quantification (LOQ) — is placed in doubt, with the further implication that the dataset may fall short of Good Laboratory Practice (GLP) requirements, potentially rising to the level of a material regulatory misrepresentation.

The reconstruction presented here further relies on the Australian TGA report, which explicitly identifies *2-hexyldecanoic acid* as the relevant metabolite, as a documentary counterpoint suggesting that the authentic catabolite was known and characterizable. Within this framework, the appearance of a different isomer in primary regulatory dossiers for an injectable medicinal product destined for millions is interpreted as functionally compatible with a strategy aimed at attenuating or even concealing the perceived

toxicological burden associated with the H410 classification — an outcome that, in a regulatory setting, is difficult to view as anything other than an affront to compliance and to the fundamental duty to safeguard public health. It is further argued that essential investigations on chronic toxicity and bioaccumulation — precisely those implicated by an H410-classified, persistent compound — were not performed or, at minimum, are not transparently documented in the reviewed material. A substance described as persistent in environmental and biological contexts would, under normal regulatory policy, demand a strengthened evidentiary package; instead, the dossier trajectory appears to have been buffered by recourse to an unstudied fatty acid that is not only presented as a reference material of unclear scientific standing, but is also inconsistent with the expected chemistry of hydrolysis of the parent molecule. Such a posture is fundamentally incompatible with transparency obligations and with the stated purpose of safeguarding public health. If the authentic metabolite is *2-hexyl* while the documentation is instead anchored to a *6-hexyl* construct (lacking H410 classification and presented as theoretically more degradable), then the reviewing authorities may have been placed in a position to evaluate an incomplete or distorted chronic-risk profile. Under that premise, the mechanisms discussed in this work — mitochondrial inhibition and nuclear-envelope destabilization — translate into undeclared, insufficiently tested long-term liabilities for exposed populations. The uncritical adoption of "6-hexyldecanoic acid" as the primary metabolite in the EMA Assessment Report (2021, p. 48) further suggests that traceability checks in international chemical repositories were not applied with the diligence expected from the CHMP (*Committee for Medicinal Products for Human Use*) quality-control framework.

In conclusion, on the basis of the evidentiary and mechanistic elements set out above, a precautionary revocation of the Conditional Marketing Authorization (CMA) for Comirnaty is proposed as a proportionate measure to restore regulatory integrity and to stop immediately any avoidable public-health exposure while the outstanding toxicological criticalities are addressed under full analytical traceability.

The grounds supporting this request can be summarized as follows:

1. *Vitiation of regulatory consent*: The EMA, as reflected in the Assessment Report of 19 February 2021, appears to have been influenced by the inclusion and subsequent endorsement of non-traceable (and potentially non-existent) chemical isomers, thereby compromising the evaluative process and plausibly underestimating risk.
2. *Public-health risk*: The intracellular persistence and bioenergetic impact of the catabolites (*2-hexyldecanoic acid* and the trialkanolamine *[(4-hydroxybutyl)azanediyl]dihexanol*) entail undeclared risks of systemic toxicity and genomic instability for exposed individuals.
3. *Safety protocols and the Precautionary Principle*: The deployment of lipid nanomaterials whose toxicological profiles are inadequately characterized is inconsistent with the Precautionary Principle and with European regulatory expectations for nanomaterial-containing medicinal products, thereby impairing recipient safety.
4. *Ethical profile*: The documented criticalities raise a manifest concern with respect to foundational standards for human experimentation, including those captured by Article 10 of the Nuremberg Code: "*During the course of the experiment the scientist in charge must be prepared to terminate the experiment at any stage, if he has probable cause to believe, in the exercise of the good faith, superior skill and careful judgment required of him, that a continuation of the experiment is likely to result in injury, disability, or death to the experimental subject.*"

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The author declares that he has not received any funding to influence what he says here and that the research was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

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