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Chemical-Physical Criticality and Toxicological Potential of Lipid Nanomaterials Contained in a COVID-19 mRNA Vaccine

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Abstract

The medicinal preparation called *Comirnaty* by Pfizer-BioNTech is an aqueous dispersion of lipid nanomaterials, intended to constitute, after thawing and dilution, the finished product for intramuscular injection. In the present study, we examine some evident chemical-physical criticalities of the preparation, regarding the manifest instability of its qualitative-quantitative composition, as well as its consequent toxicological potential, in this case related to the possible formation of ROS (*reactive oxygen species*), after intramuscular inoculation, in different biological sites, such as, potentially, kidneys, liver, heart, brain, etc., causing dysfunctions and alterations thereof. Of particular concern is the presence in the formulation of the two functional excipients, ALC-0315 and ALC-0159, never used before in a medicinal product, nor registered in the European Pharmacopoeia, nor in the European C&L inventory. The current Safety Data Sheets of the manufacturer are omissive and non-compliant, especially with regard to the provisions of current European regulation on the registration, evaluation, authorization and restriction of nanomaterials. The presence of electrolytes in the preparation and the subsequent dilution phase after thawing and before inoculation raise well-founded concerns about the precarious stability of the resulting suspension and the polydispersity index of the nanomaterials contained in it, factors that can be hypothesized as the root causes of numerous post-vaccination adverse effects recorded at statistical-epidemiological levels. Further immediate studies and verifications are recommended, taking into consideration, if necessary and for purely precautionary purposes, the immediate suspension of vaccinations with the Pfizer-BioNTech Comirnaty preparation.

Keywords: *COVID-19 mRNA vaccine, LNP, lipid nanoparticles, nanomaterials, nanoforms, electrolytes, reactive oxygen species, aggregate, agglomerate*

INTRODUCTION

The medicinal product called Comirnaty COVID-19 mRNA BNT162b2 is a concentrated semi-finished product, based on a particular strand of mRNA encapsulated in lipid nanoparticles (LNPs), and intended to constitute, after the phases of thawing and dilution with sodium chloride solution, a dispersion of nanomaterials injectable intramuscularly. It was placed on the market in Europe, with *conditional marketing authorisation* issued by EMA (European Medicines Agency) on 21 December 2020 and first Assessment Report on 19 February 2021 (EMA/707383/2020, 2021).

Nanomaterials (also called *nanoparticles* or *nanoforms*) are defined and described by ECHA (*European Chemicals Agency*) as follows (with my emphasis in italics added here and throughout the remaining quoted entries in this paper):

> Nanomaterials are chemical substances or materials with *particle sizes between 1 to 100 nanometers* in at least one dimension.[1]
>
> Due to an increased specific surface area by volume, nanomaterials may have *different characteristics compared to the same material without nanoscale features*. As a result, *the physicochemical properties of nanomaterials may differ from those of bulk substances or particles of a larger size*.
>
> Many everyday products containing nanomaterials are already on the European market such as batteries, coatings, anti-bacterial clothing and cosmetics. While nanomaterials may offer technical and commercial opportunities, *they may also pose risks to our health and the environment*. Just like any other substance on the EU market, *it is important to ensure that their uses are properly assessed and that any risks are adequately controlled*.

Already in 2011, the European Commission published a "Recommendation" containing the definition of nanomaterial, inviting member states, union agencies and economic operators to use it in the adoption and implementation of legislation and strategic and research programs related to nanotechnology products, in particular by making appropriate amendments in several European regulations, including Regulation (EC) No 1907/2006 (REACH) and Regulation (EC) No 1272/2008 (CLP), in order to harmonize the way nanomaterials were defined in the different legal frameworks.

This Recommendation was subsequently accepted and included in the Commission Regulation (EU) 2018/1881 entered into force on January 1, 2020, which, in addition to introducing some substantial changes to the REACH Regulation, set out a much more articulated and complete definition of nanomaterial, introducing specific indications for registration, evaluation, authorization and restrictions concerning the so-called *nanoforms*.

On page 8 of this Regulation we read:

> *Definition of a nanoform and a set of similar nanoforms:*
>
> On the basis of the Commission Recommendation of 18 October 2011 on the definition of nanomaterial, *a nanoform is a form of a natural or manufactured substance containing particles, in an unbound state or as an aggregate or as an agglomerate* and where, for 50% or more of the particles in the number size distribution, *one or more external dimensions is in the size range 1 nm-100 nm*, including also by derogation fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm. For this purpose, "particle" means a minute piece of matter with defined physical boundaries; "agglomerate" means a collection of weakly bound particles or aggregates

[1] It is important to realize that 1 nanometer = 1 billionth of a meter = 1 millionth of a millimeter, a size that is tens of thousands of times smaller than the thickness of a human hair.

> where the resulting external surface area is similar to the sum of the surface areas of the individual components and "aggregate" means a particle comprising of strongly bound or fused particles.

Why is it important to characterize and distinguish between the various types of nanoforms, such as individual particles and their possible "aggregates" or "agglomerates"? It should be emphasized that the criteria for assessing the hazard and toxicity of nanoforms are substantially those related to their *size*. In fact, in the above legally binding definition, there is no reference to the chemical composition (organic or inorganic) of the material under consideration, but only to the *size of the particles* that constitute it, whether of *natural, derived or synthetic origin*. In particular, in order to assess their toxicological profile, first of all the *chemical-physical characteristics*, and in particular the *size* of the particles, their numerical *size distribution*, their *shape* and other *morphological parameters* (such as crystallinity, information on the whole nanometric assembly, including for example shell structures or hollow structures, etc.), their *surface area* (volume-specific, mass-specific area, or both) must be taken into account, as well as their *molecular structures* (EU Commission Reg. 2018, p. 10).

COMPOSITION AND NANOMATERIALS OF THE COMIRNATY COVID-19 mRNA VACCINE BNT162B2

As is now well known, the Pfizer-BioNTech COVID-19 vaccine, generally called "Comirnaty BNT162b2", contains a particular strand of *mRNA encapsulated in lipid nanoparticles*. These nanoparticles have the primary function of protecting mRNA from enzymatic degradation and thus allowing its penetration into the cells of the host organism, after intramuscular injection (Nance & Meier, 2021).

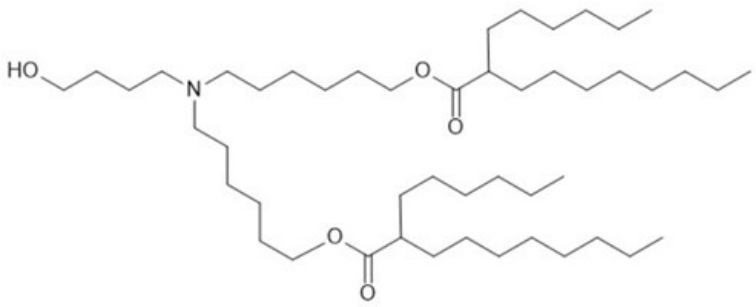

Figure 1. Molecular structure of ALC-0315.

In the formulation, *four specific lipid components* are distinguished, capable of forming, in combination with each other, nanoparticles dispersed in an aqueous medium:

a) *ALC-0315* (ionizable, *cationic* functional lipid). Chemical name: ((4-hydroxybutyl (azanediyl) bis (hexane-6,1-diyl) bis (2-hexyldecanoate)). CAS No. 2036272-55-4. Amphiphilic molecule [2], of synthetic origin, consists of a tertiary amine structure with a hydroxy-butyl group and two exilic groups esterified with 2-hexyldecanoic acid (Figure 1).

[2] A molecule is called *amphiphilic* (also amphipathic) when it contains both a hydrophilic group (water-loving, polar) and a lipophilic group (fat-loving, non-polar).

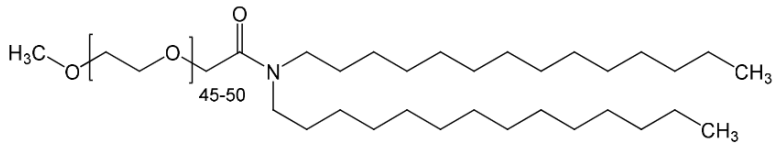

Figure 2. Molecular structure of ALC-0159.

b) *ALC-0159* (functional lipid, non-ionic, *polyethoxylated*). Chemical name: 2 ([polyethylene glycol]-2000)-N,N-ditetradecylacetamide. CAS No. 1849616-42-7. Amphiphilic molecule, of synthetic origin, consisting of a di-myristil-amide of hydroxyacetic acid, polyethoxylated with 45/50 moles of ethylene oxide (Figure 2).

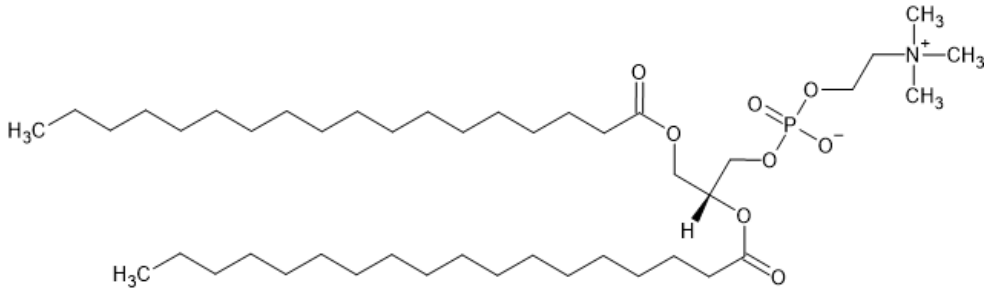

Figure 3. Molecular structure of DSPC.

c) *DSPC* (structural support phospholipid, "*helper lipid*"). Chemical name: 1,2-Distearoyl-sn-glycero-3-phosphocholine. CAS No. 816-94-4. Molecule of semi-synthetic origin, amphiphilic, consisting of a phosphoglyceride in which one group is phosphatidylcholine and two groups are stearic acid chains (18:0) (Figure 3).

d) *Cholesterol* (lipid having functions, in this case, of structural support). Organic molecule belonging to the class of sterols. CAS No. 57-88-5.

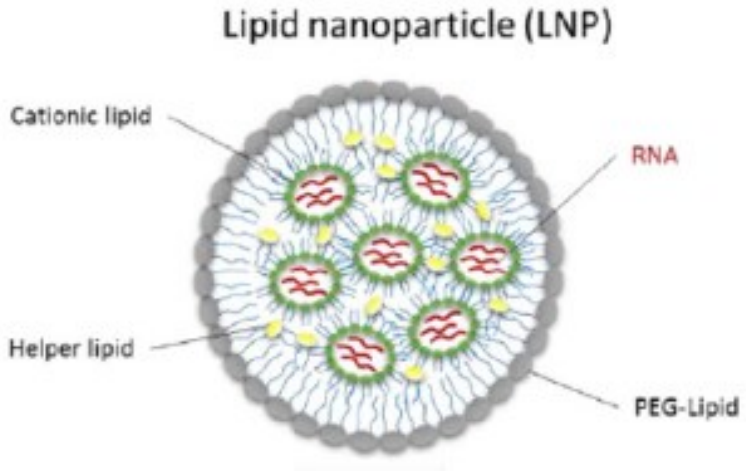

Figure 4. Schematic representation of the structure of a Pfizer-BioNTech Comirnaty Vaccine nanoparticle (EMA/594686/2021 p. 15).

These four lipid components constitute the fundamental excipients of Comirnaty, instrumental to the formation of lipid spheroidal nanoforms (Tenchov et al. 2021), i.e. lipid nanoparticles (LNPs)

of the type schematically represented in Figures 4 and 5, and intended to encapsulate, incorporate, protect and convey the active substance, consisting of mRNA BNT162b2.

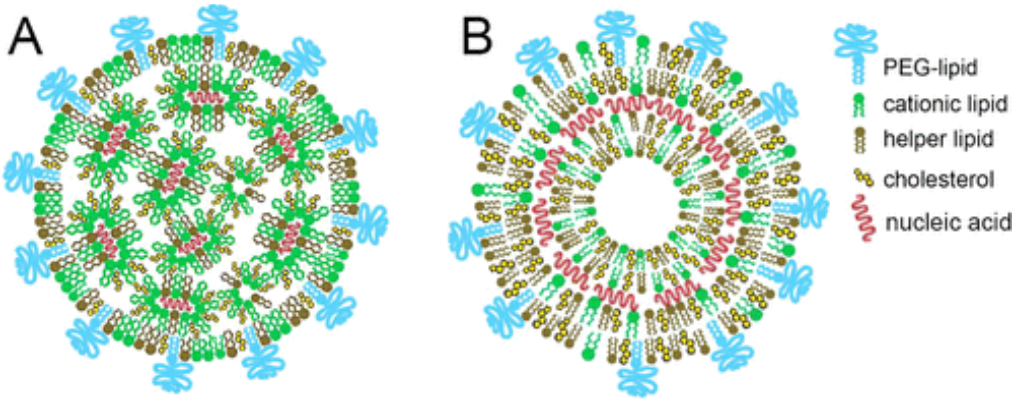

Figure 5. Suggested structures of lipid nanoparticle nucleic acid carriers: nucleic acids organized in inverse lipid micelles inside the nanoparticle (A); nucleic acids intercalated between the lipid bilayers (B) (Tenchov et al. 2021).

As stated by EMA in the aforementioned Comirnaty Assessment report of 19 February 2021 (EMA/707383, 2021), the nanoparticles, formed by the four lipids as described above, are solid particles, held in suspension in an aqueous medium and in the presence of the so-called *Phosphate Buffered Saline* (consisting of *inorganic electrolytes*), which maintains the pH at values between 6.9 and 7.9, and a sugar (sucrose), as a cryoprotective agent.

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

Assessment report
Comirnaty
Common name: COVID-19 mRNA vaccine (nucleoside-modified)
Procedure No. EMEA/H/C/005735/0000
19 February 2021
EMA/707383/2020 Corr.1
Committee for Medicinal Products for Human Use (CHMP)

Specific Obligation to complete post-authorisation measures for the conditional marketing authorisation

This being a conditional marketing authorisation and pursuant to Article 14-a of Regulation (EC) No 726/2004, the MAH shall complete, within the stated timeframe, the following measures:

In order to confirm the purity profile and ensure comprehensive quality control and batch-to-batch consistency throughout the lifecycle of the finished product, the MAH should provide additional information about the synthetic process and control strategy for the excipient ALC-0315.	July 2021. Interim reports: January 2021, April 2021.
In order to confirm the purity profile and ensure comprehensive quality control and batch-to-batch consistency throughout the lifecycle of the finished product, the MAH should provide additional information about the synthetic process and control strategy for the excipient ALC-0159.	July 2021. Interim reports: January 2021, April 2021.
In order to confirm the efficacy and safety of Comirnaty, the MAH should submit the final Clinical Study Report for the randomized, placebo-controlled, observer-blind study C4591001.	December 2023

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Figure 6. Assessment Report EMA/Pfizer-BioNTech Comirnaty, February 19, 2021, page 140.

And, with regard to the two functional lipid ingredients, we read that, since the marketing *authorization is subject to conditions* (EMA/707383, p. 140), the holder of this authorization (Pfizer-

BioNTech) must complete, within the established timeframe, some specific tasks. Among these: "*In order to confirm the purity profile and ensure comprehensive quality control and batch-to-batch consistency throughout the lifecycle of the finished product, the MAH [3] should provide additional information about the synthetic process and control strategy*" for both "new" lipid excipients *ALC-0315* and *ALC-0159*. The expiry date of the authorization for the delivery of this information is by July 2021, with interim reports scheduled for January 2021 and April 2021. The final report on the clinical study "*to confirm the efficacy and safety of Comirnaty*" is expected and required by December 2023 (Figure 6).

As is now known, at the date of this writing, the contents of the reports, presumably submitted by the authorization holder within the scheduled dates (January 2021, April 2021, July 2021), have been kept classified and undisclosed by EMA. For this reason, some inevitable and pressing questions arise: does the additional information on the *synthesis process and the control strategy*, provided in the interim reports, contain (or not) the evidences required by European legislation regarding the registration and authorization of *nanoforms*? In other words, has all the information on the *chemical-physical and toxicological characteristics of the nanoforms of the medicinal product Comirnaty* been provided? And, if so, why keep it secret if it is obligatory by European law for *every* nanoform commercialized in the European Community?

REGULATORY NON-COMPLIANCES AND ABSENCE OF TOXICOLOGICAL STUDIES

LACK OF REGISTRATION IN PHARMACOPOEIA

All the ingredients of the medicinal product *Comirnaty* are *known in the European Pharmacopoeia, except ALC-0315 and ALC-0159*. Both these nanomaterials are classified by EMA as "novel excipients" as "never previously used in a medicinal product in Europe" and "not registered in the EU Pharmacopoeia" (EMA/707383, p. 23).

It is disconcerting to see that a medicinal product that has been manufactured, authorized and administered in billions of doses contains ingredients that have never been registered in the Pharmacopoeia. The significance and gravity of such an omission is understood by reading the description of the *Purpose of the European Pharmacopoeia*:

> The European Pharmacopoeia is a single reference work for the quality control of medicines in the signatory states of the Convention on its elaboration.
>
> The official standards published within provide a *legal and scientific basis for quality control during the development, production and marketing processes*.
>
> They concern the qualitative and quantitative composition and the tests to be carried out on medicines, on the raw materials used in production of medicines and on the intermediates of synthesis. *All producers of medicines and/or substances for pharmaceutical use must therefore apply these quality standards in order to market their products in the signatory states of the Convention* […]
>
> *The purpose of the European Pharmacopoeia is to promote public health by the provision of recognized common standards for the quality of medicines and their components.* Such standards are to be *appropriate as a basis for the safe use of medicines by patients*. In addition, their existence facilitates the free movement of medicinal products in Europe and beyond.
>
> *European Pharmacopoeia monographs and other texts are designed to be appropriate to the needs of:*

[3] Marketing Authorization Holder.

- *regulatory authorities;*
- *those engaged in the quality control of medicinal products and their constituents;*
- *manufacturers of medicinal products and their individual components.*

> The European Pharmacopoeia is widely used internationally. As globalization and expansion in international trade present a growing need to develop global quality standards for medicines, the Commission works closely with all users of the Pharmacopoeia worldwide. [my emphasis] (EU Pharmacopoeia, 2023).

OMISSIVE AND NON-COMPLIANT SAFETY DATA SHEETS

In addition to being unknown to the European Pharmacopoeia, the two lipid components ALC-0315 and ALC-0159 are not even reported in the C&L inventory.[4] Consequently, they do not have a REACH registration number and their CLP classification is not known. In other words, their general toxicological profile is not officially known — neither as substances, nor as nanoforms made up of them. This is also confirmed by what is stated in section 3 (*Composition/Ingredient Information*) of the Pfizer-BioNTech COVID-19 vaccine Product Safety Data Sheet, dated 7 December 2021 (Figure 7), where, under the heading *Classification according to Regulation (EC) No 1272/2008 (CLP)* appears the note "No data available", and under the heading *REACH Registration Number*, no number appears.

SAFETY DATA SHEET

Product Name Pfizer-BioNTech COVID-19 Vaccine
Revision date 07-Dec-2021

https://safetydatasheets.pfizer.com

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Section 3: COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous

Chemical name	Weight-%	REACH Registration Number	EC No	Classification according to Regulation (EC) No. 1272/2008 [CLP]	Specific concentration limit (SCL)	M-Factor	M-Factor (long-term)
ALC-0315 2036272-55-4	< 2		Not Listed	No data available	Not Listed	No data available	No data available
ALC-0159 1849616-42-7	< 1		Not Listed	No data available	Not Listed	No data available	No data available

Figure 7. Safety Data Sheet, section 3 dated 7 December 2021 of the Pfizer-BioNTech *COVID*-19 vaccine.

This contrasts with what we read on the official website of the European Union (*Your Europe*):

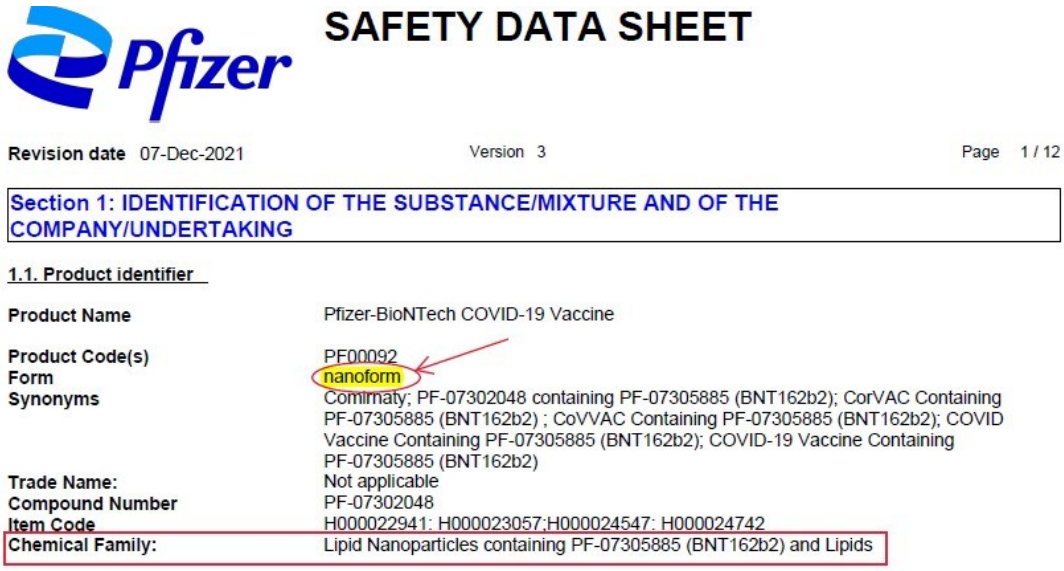
> If you manufacture or import one ton or more per year of a chemical substance in the EEA, you must record this in the REACH database. REACH stands for the Registration, Evaluation, Authorisation and Restriction of Chemicals.
>
> REACH applies to all chemical substances, both those needed for industrial processes and those we use in our everyday lives, in paints, cleaning products, clothes, furniture and electrical appliances, for example. It thus affects most businesses in the European Economic Area (EEA).
>
> *Non-registered substances must not be marketed or used.* [my emphasis]

[4] The C&L inventory is a database managed by ECHA that contains information on the classification and labelling of substances placed on the European market. This database includes information on notified and registered substances, but also the list of harmonised classifications and labelling according to Annex VI of the CLP Regulation https://echa.europa.eu/information-on-chemicals/cl-inventory-database.

The characteristics of the particles specifying the nanoform, as required and described in Annex VI of the REACH Regulation, should also be indicated in the Safety Data Sheet of the manufacturer:

> If the substance is registered and it covers a nanoform, *the particle characteristics that specify the nanoform*, as described in Annex VI, *shall be indicated.*
>
> *If the substance is not registered, but the safety data sheet covers nanoforms, the particle characteristics of which have impact on the safety of the substance, those characteristics shall be indicated.* [my emphasis] (EU Commission Reg. 2020/878, p.34).

On the contrary, although it is expressly indicated, in section 1 of the same Pfizer-BioNTech Safety Data Sheet, that the product is a *nanoform* and belongs to the Chemical Family called *lipid nanoparticles*



SAFETY DATA SHEET

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Section 1: IDENTIFICATION OF THE SUBSTANCE/MIXTURE AND OF THE COMPANY/UNDERTAKING

1.1. Product identifier

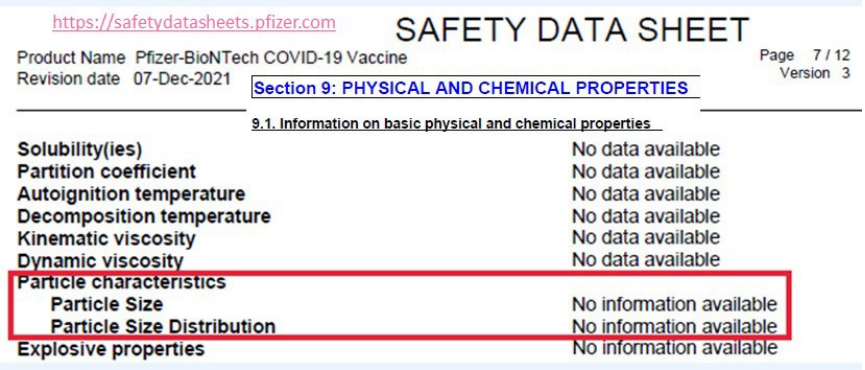
Product Name	Pfizer-BioNTech COVID-19 Vaccine
Product Code(s)	PF00092
Form	nanoform
Synonyms	Comirnaty; PF-07302048 containing PF-07305885 (BNT162b2); CorVAC Containing PF-07305885 (BNT162b2) ; CoVVAC Containing PF-07305885 (BNT162b2); COVID Vaccine Containing PF-07305885 (BNT162b2); COVID-19 Vaccine Containing PF-07305885 (BNT162b2)
Trade Name:	Not applicable
Compound Number	PF-07302048
Item Code	H000022941; H000023057;H000024547; H000024742
Chemical Family:	Lipid Nanoparticles containing PF-07305885 (BNT162b2) and Lipids

Figure 8. In section 1.1 of the Pfizer-BioNTech Safety Data Sheet, dated 7 December 2021, version 3, the nanoform configuration of the product is expressly indicated.

containing PF-07305885 (BNT162b2) and Lipids (Figure 8), the *characteristics of the nanomaterial present in the composition are not reported in any other section* of the document, as opposed to the dictates of Regulation (EU) 2020/878, which clearly prescribe, on page 45, how the characteristics of nanoforms must be reported in subsection 9.1 of the Safety Data Sheet, as follows:

> […] (r) *Particle characteristics:*
>
> Only apply to solids.
>
> The *particle size* (median equivalent diameter, method of calculation of the diameter (number-, surface- or volume-based) and the range in which this median value varies), shall be indicated. Other properties may also be indicated, such as size distribution (e.g. as a range), shape and aspect ratio, *aggregation and agglomeration state*, specific surface area and dustiness. *If the substance is in nanoform or if the mixture supplied contains a nanoform, those characteristics shall be indicated in this subsection, or referred to if already specified elsewhere in the safety data sheet.* [my emphasis]

Subsection 9.1 of the Pfizer-BioNTech Safety Data Sheet is shown in Figure 9. Under *Particle characteristics*, it reads surprisingly: *No information available.*



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Section 9: PHYSICAL AND CHEMICAL PROPERTIES

9.1. Information on basic physical and chemical properties

Solubility(ies)	No data available
Partition coefficient	No data available
Autoignition temperature	No data available
Decomposition temperature	No data available
Kinematic viscosity	No data available
Dynamic viscosity	No data available
Particle characteristics	
Particle Size	No information available
Particle Size Distribution	No information available
Explosive properties	No information available

Figure 9. Section 9.1 of the Safety Data Sheet, version 3, dated 7 December 2021, of the Pfizer-BioNTech Comirnaty Vaccine.

NO CARCINOGENICITY, GENOTOXICITY AND MUTAGENICITY STUDIES

The analysis of the characteristics of nanoparticles (size, total surface area, state of aggregation or agglomeration, polydispersity index, surface charge, etc.), as already described above and as expressly reiterated in the aforementioned regulations, is essential in order to determine their possible cytotoxic, genotoxic, mutagenic and carcinogenic potential. The *state of agglomeration*, in particular, can in itself represent an important risk factor, as it can affect not only the *translocation of nanomaterials in or through various organs* and tissues, but also the *degree of accumulation* within those tissues and, consequently, the related *catabolic elimination processes.* (Bruinink et al., 2015). Despite this, EMA, in its report dated 19 February 2021, regarding the assessment of the Comirnaty vaccine, writes:

> No genotoxicity nor carcinogenicity studies have been provided. The components of the vaccine formulation are lipids and RNA that are *not expected to have genotoxic potential.* (EMA/707383, 2021, p. 55)
>
> As per guidance, no genotoxicity nor carcinogenicity studies were performed. The components of the vaccine (lipids and mRNA) are *not expected to have genotoxic potential.* This is acceptable to the CHMP. [5] [my emphasis] (EMA/707383, 2021, p. 56).

Note how, in expressing a hypothetical absence of genotoxicity and carcinogenicity of the lipid components of the Comirnaty vaccine, EMA seems to ignore that the two *novel excipients* ALC-0315 (ionizable, cationic) and ALC-0159 (non-ionic, polyethoxylated) are not simple "lipids", but, as additionally and widely described in other sections of the same EMA report, they are "functional" lipids, that is to say fundamental and determinant in order to carry out the formation, in situ, of *lipid nanoparticles*, that is, substances subjected to all the aforementioned European provisions and regulations on the registration, evaluation, authorization and restriction of *Nanomaterials*. Note that these are provisions and regulations including, among other things, the *obligation for manufacturers to provide ALSO the appropriate genotoxicity and carcinogenicity tests specifically prescribed for nanoforms.*

In particular, as stated on page 6 of the aforementioned Regulation (EU) 2018/1881 concerning nanoforms:

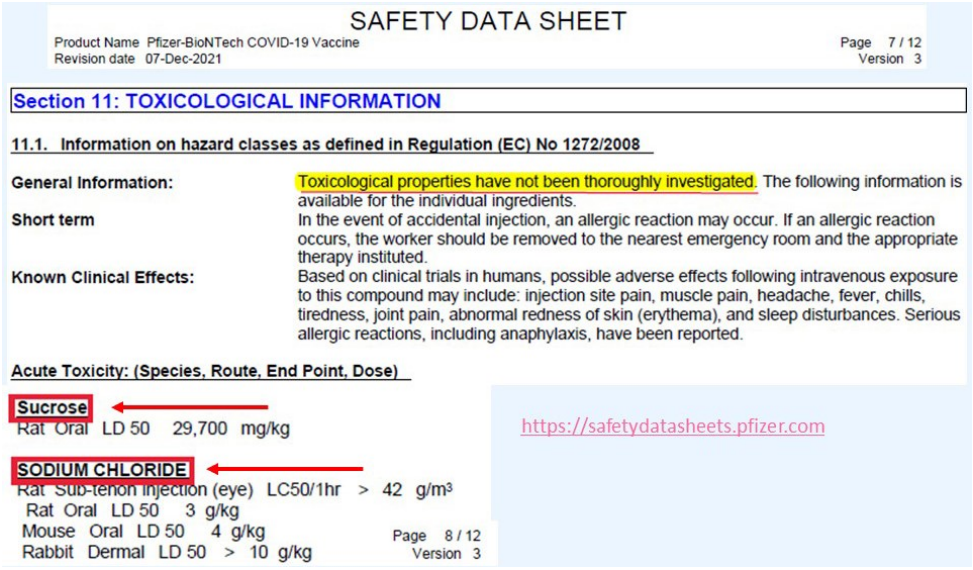
> The assessment should always include a statement as to whether the substance or, when applicable, *nanoforms* thereof fulfils or does not fulfil the criteria given in Regulation (EC) No

[5] CHMP: European Committee for Medicinal Products for Human Use.

> 1272/2008 for classification in the hazard class *carcinogenicity* category 1A or 1B, in the hazard class germ cell *mutagenicity* category 1A or 1B or in the hazard class *reproductive toxicity* category 1A or 1B. [my emphasis]

It is now universally established that among the greatest risks to human health caused by the exceptional penetrability and mobility of nanoforms within biological systems, those related to genotoxicity and carcinogenicity must be taken into account. The related in vitro assays are considered an extremely important, if not indispensable, tool for a thorough understanding of the toxicity mechanisms and an a*dequate assessment of the health risks caused by nanomaterials, especially in the medium to long term* (Barone et al., 2017).

Equally non-compliant, and in conflict with the now consolidated regulatory-toxicological practice relating to nanoforms, Section 11 (Toxicological information) of the Pfizer-BioNTech Safety Data Sheet, with reference to the Comirnaty product says: *Toxicological properties have not been thoroughly investigated* (Figure 10). The only toxicological information reported in this section is that relating to the individual components, including, for example, the toxicological profiles of sugar (sucrose) and common table salt (sodium chloride), but excluding those of the aforementioned nano-functional lipids ALC-0315 and ALC-0159. Also, there is no mention, in that section, of nanomaterials in the composition, nor is there any reference to the toxicological assays required by law on nanoforms.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Section 11: TOXICOLOGICAL INFORMATION

11.1. Information on hazard classes as defined in Regulation (EC) No 1272/2008

General Information: Toxicological properties have not been thoroughly investigated. The following information is available for the individual ingredients.

Short term In the event of accidental injection, an allergic reaction may occur. If an allergic reaction occurs, the worker should be removed to the nearest emergency room and the appropriate therapy instituted.

Known Clinical Effects: Based on clinical trials in humans, possible adverse effects following intravenous exposure to this compound may include: injection site pain, muscle pain, headache, fever, chills, tiredness, joint pain, abnormal redness of skin (erythema), and sleep disturbances. Serious allergic reactions, including anaphylaxis, have been reported.

Acute Toxicity: (Species, Route, End Point, Dose)

Sucrose
Rat Oral LD 50 29,700 mg/kg

https://safetydatasheets.pfizer.com

SODIUM CHLORIDE
Rat Sub-tenon injection (eye) LC50/1hr > 42 g/m³
Rat Oral LD 50 3 g/kg
Mouse Oral LD 50 4 g/kg
Rabbit Dermal LD 50 > 10 g/kg

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Figure 10. Section 11 (Toxicological Information) of the Pfizer-BioNTech Safety Data Sheet, version 3, dated 7 Dec 2021.

REACTIVE OXYGEN SPECIES (ROS) FORMATION AND NANOPARTICLE TOXICITY

It is also important to note that the main lipid component in the Pfizer-BioNTech formulation, ALC-0315, being made up of a tertiary amine, tends to be protonated in a moderately low pH environment, thus giving rise to the formation of *cationic nanoforms*, i.e. having a *positive surface charge*. In fact, it is thanks to the attraction with the portions of the mRNA having negative electric charge that the formation of spheroidal nanoforms takes

place, such as those illustrated in Figure 5. What seems to be ignored in EMA's Assessment Reports and Pfizer-BioNTech Safety Data Sheets is that experimental data show that *cytotoxic and genotoxic effects are enhanced if nanoparticles have a positive charge* (Barone et al., 2017; Fröhlich, 2012).

Nanoparticles consisting of monovalent cationic lipids, such as ALC-0315, have in fact been shown to be significantly more efficient in inducing cell death through the production of *reactive oxygen species* (ROS).

It is now confirmed by numerous studies that the toxic effects produced by nanoparticles in biological systems are mainly and substantially due to the formation of ROS inside cells. ROS are particles that contain oxygen, among which the most relevant are *hydrogen peroxide* (H_2O_2), *superoxide anion radical* (O_2^-) and *hydroxyl radicals* (•OH).

They are predominantly produced in cellular organelles such as the endoplasmic reticulum (ER), peroxisomes, and particularly in mitochondria. Nanoparticles containing monovalent cationic lipids have been widely used in anticancer therapies for the administration of nucleic acids such as *si*RNA and polypeptides, directly into target cells. However, several studies have shown that cationic liposomes induce ROS formation and ROS-mediated toxicity in healthy cells and, at the same time, reduce cell viability. For example, depending on lipid concentration, surface density of cationic lipids and particle size, nanoparticles containing cationic lipids can lead to ROS generation and death of *HepG2* liver cancer cells (Yun et al., 2016).

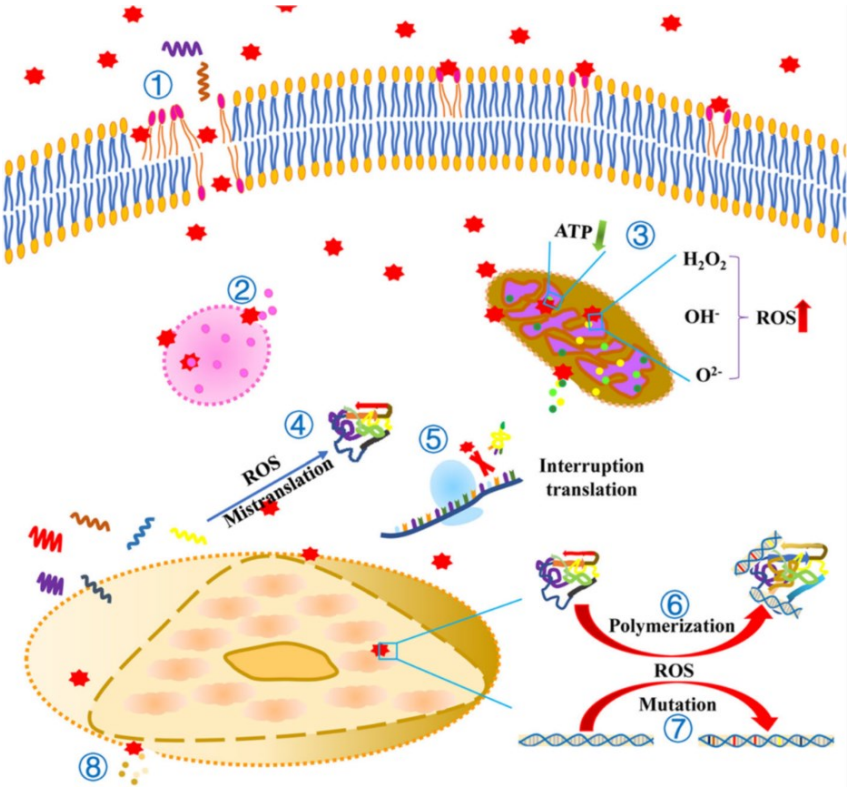


Figure 11. "Cellular events induced by Nanoparticles (NPs). ① NPs contribute to the destruction of the cell membrane and to lipid peroxidation. ② The lysosomal membrane is destroyed by NPs and results in the release of their contents. ③ The mitochondrial membrane is damaged by NPs, leading to content release. NPs reduce the generation of ATP and increase the production of ROS. ④ The ROS induced by NPs results in the mistranslation of RNA. ⑤ NPs prevent the binding of tRNA to the ribosome. ⑥ The ROS induced by NPs result in the polymerization of proteins and DNA. ⑦ The ROS induced by NPs leads to DNA mutations ⑧ The nuclear membrane is destroyed by NPs, resulting in the release of its contents" (Yu et al., 2020).

In almost all scientific studies on the subject, it is noted that, despite the undoubted benefits and progress made in the use of nanomaterials in the biomedical field, concerns remain about the potential toxicological effects of nanoparticles, especially in relation to their tendency to generate reactive oxygen species. Due to their strong oxidation potential, excess ROS induced by nanoparticles can cause damage to biomolecules and cell organelle structures. They can produce oxidative carbonylation of proteins, lipid peroxidation, DNA/RNA breakdown, and destruction of cell membranes, factors that can induce a complex of pathophysiological effects, such as genotoxicity, necrosis, apoptosis, cytokine inflammation, fibrosis, metaplasia, hypertrophy, carcinogenicity, or even mutagenesis impacting future generations (Yu et al., 2020; Figure 11). Furthermore, Yu et al. point out that the *extreme penetration and mobility of nanoparticles* within the body account for their *easy entry into the systemic circulation* and *accumulation in organs such as kidneys, liver, heart, brain, intestinal tract, and lungs, causing dysfunctions and alterations*.

There is now overwhelming evidence that overproduction of ROS is the main cause of nanoparticle biotoxicity. By concentrating mainly in lysosomes, mitochondria, and the nucleus of the cell, and generating ROS at those sites, nanoparticles can cause devastating consequences. Numerous studies irrefutably confirm that nucleotides components of cellular DNA and RNA constitute a significantly vulnerable target to the aggression of ROS generated by nanomaterials. (Imlay et al., 1988; Maki et al., 1992; Demple et al., 1994).

This can result in irreparable genetic damage, resulting in the development of *genotoxicity*, (Kang et al., 2008; Singh et al., 2009; Chompoosor et al., 2010; Di Bucchianico et al., 2013; Proquin et al., 2017), *mutagenicity* (Kirsch-Volders et al., 2002; Mateuca et al., 2006; Dufour et al., 2006; Levine et al., 2017; Jena, 2012), *carcinogenicity* (Rusyn et al., 2004; Nel et al., 2006; Liou et al., 2010; Tretyakova et al., 2015).

The accumulation of nanoparticles in the body can further induce inflammation and immune responses, which in turn cause cell injury or *apoptosis* (cell death), *dysfunction of vital organs* and, finally, stimulate the onset of numerous diseases, such as *Alzheimer's, Parkinson's, inflammation of the liver*, and *dysembryoplasia*. (Yu et al., 2020, p. 9)

CHEMICAL-PHYSICAL CRITICALITIES OF NANOFORMS AND CONSEQUENT TOXICOLOGICAL RISKS

The Polydispersity Index (PI)

As already mentioned, nanoparticles inserted in a dispersing medium, such as an aqueous solution as in the Comirnaty preparation, tend to form *aggregates* or *agglomerates of different shapes and sizes*, thus modifying their original dimensional characteristics, and, consequently, all those parameters crucial for the evaluation of their toxicological profile (Figure 12). A fundamental parameter to which both toxicologists and the European legislator assign great importance is definitely the *degree of agglomeration/aggregation* (called *Polydispersity index*) of nanoparticles in an aqueous medium.

The *Polydispersity index* (PI) is a measure of the heterogeneity of a sample size of that nanomaterial (Figure 13). Its value is included between 0 and 1: the closer it is to 0 the more the suspension is *monodisperse* (uniform), while for indices close to 1 the suspensions are considered totally *poly-dispersed* (non-uniform). International standardization organizations (ISOs) have established that PI values < 0.05 are specific to monodisperse samples, while values > 0.7 are related to distribution of large

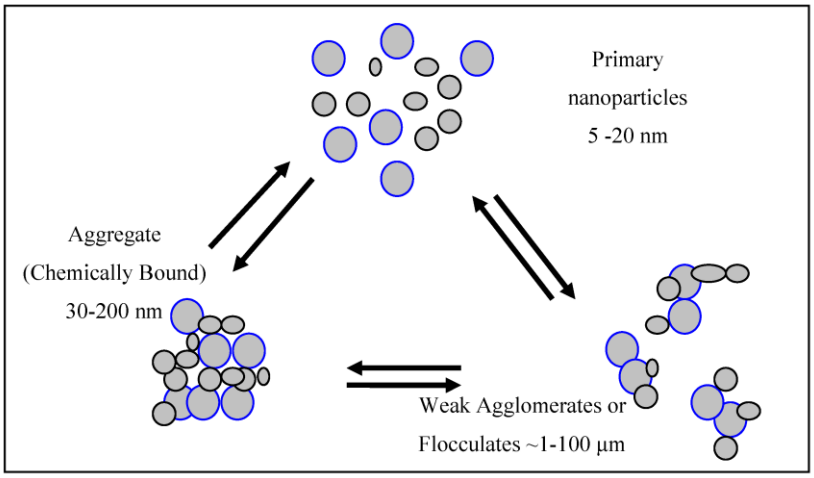


Figure 12. Formation of nanoparticle aggregates and agglomerates.

particles (polydisperse). In general, a suspension can be considered *monodisperse* for PI values ≤ 0.2, on average polydisperse for 0.2 ≤ PI ≤ 0.5 and *polydisperse* for values greater than 0.6. PI can be obtained from instruments using dynamic light scattering (DLS) [6] or electronic micrographs.

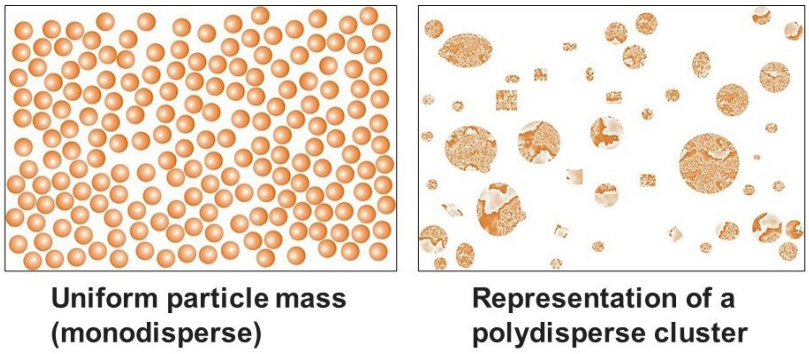


Figure 13. Monodisperse (uniform) and polydisperse (non-uniform) nanomaterial suspensions.

The fact that the toxicological profile of a given nanomaterial is directly, though not exclusively, linked to the Polydispersity index is easily understandable considering that, depending on how much the primary nanoparticles aggregate or agglomerate with each other, larger secondary nanoparticles are generated. These in turn could affect the exposure and bioavailability of the preparation in different ways. For example, if primary particles aggregate or agglomerate with each other to form larger, heterogeneous particulates with a higher PI, the material, or part of the material, may not enter a cell and/or may deposit in tissues or organs not foreseen in its primary biological fate. The heterogeneity of size distribution can, in other words, determine a considerable variability of the potential impact both on the translocation of the different aggregates, and on the penetration of biological barriers, such as crossing the blood-brain barrier, penetration into cells and subcellular structures, and on the *delivery into biological systems of any impurities or contaminants incorporated in the*

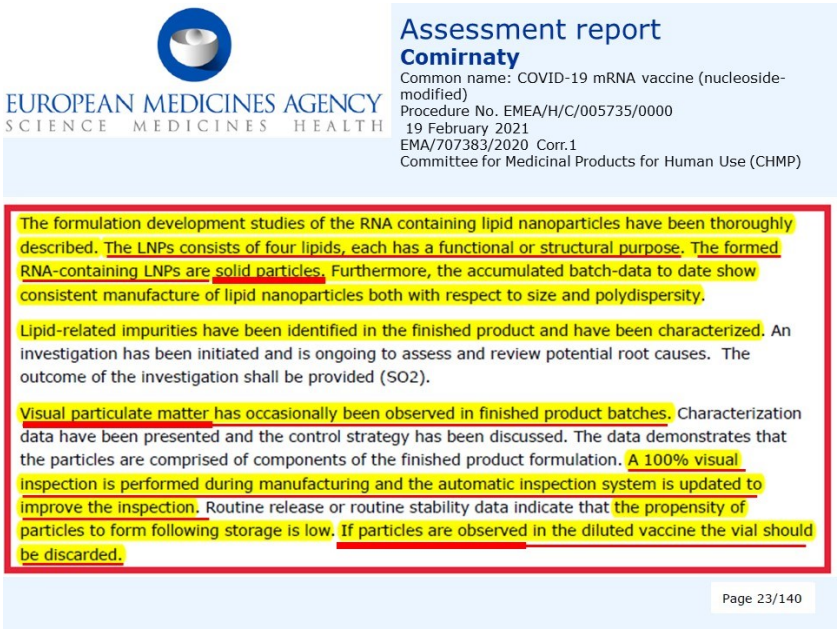
[6] ISO 22,412:2017 Particle size analysis — Dynamic light scattering (DLS)
https://www.iso.org/obp/ui/#iso:std:iso:22412:ed-2:v1:en

particulate matter, especially where such impurities or contaminants are also of toxicological significance.[7]

At this point it is evident that, if a suspension of nanoforms, of the type of that of the medicinal product Comirnaty, presented, at the time of inoculation, an index of excessive polydispersity (e.g. > 0.7), its efficacy (understood as the ability to penetrate through the cellular and subcellular membranes and release the mRNA in the endosomal district, and from there in the cytosol of the host cell) would be substantially inhibited, if not nullified. In this case we would therefore have a *totally ineffective medicinal product*, as not able to perform the immunological task of releasing the mRNA encoding the viral Spike protein inside the host cell. And, at the same time, the larger size *aggregates* or *agglomerates* (often improperly called particulates), failing to penetrate into the cells, could follow different and unexpected biological pathways or even settle in tissues from which they could be metabolized or eliminated with difficulty, while triggering at the same time possible *allergic or anaphylactic reactions* (Moghimi, 2021). An investigation published in the *British Medical Journal* in March 2021 shows that these problems have remained unresolved, raising *serious concerns about the location of such lipid nanoparticles in the body after inoculation*. It is noteworthy that, in the entire EMA report of 19 February 2021, no reference is made to the actual value of the Polydispersity index of Comirnaty lipid nanomaterials, although, on page 23, it is asserted that:

> Visual particulate matter has *occasionally* [sic] been observed in finished product batches [...] *If particles are observed in the diluted vaccine the vial should be discarded.* [Figure 14]

At this point, however, it is inevitable to ask: what does "occasionally" mean in such a pharmacological, immunological, toxicological, and regulatory context? How frequently is particulate matter observed? In which and how many batches? What were the PI values for each specific batch concerned? To which specific phase of the industrial process were these "occasional" anomalies related? Why did they happen in certain batches and not in others? What hypotheses have been formulated in order to


EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

Assessment report
Comirnaty
Common name: COVID-19 mRNA vaccine (nucleoside-modified)
Procedure No. EMEA/H/C/005735/0000
19 February 2021
EMA/707383/2020 Corr.1
Committee for Medicinal Products for Human Use (CHMP)

The formulation development studies of the RNA containing lipid nanoparticles have been thoroughly described. The LNPs consists of four lipids, each has a functional or structural purpose. The formed RNA-containing LNPs are solid particles. Furthermore, the accumulated batch-data to date show consistent manufacture of lipid nanoparticles both with respect to size and polydispersity.

Lipid-related impurities have been identified in the finished product and have been characterized. An investigation has been initiated and is ongoing to assess and review potential root causes. The outcome of the investigation shall be provided (SO2).

Visual particulate matter has occasionally been observed in finished product batches. Characterization data have been presented and the control strategy has been discussed. The data demonstrates that the particles are comprised of components of the finished product formulation. A 100% visual inspection is performed during manufacturing and the automatic inspection system is updated to improve the inspection. Routine release or routine stability data indicate that the propensity of particles to form following storage is low. If particles are observed in the diluted vaccine the vial should be discarded.

Page 23/140

Figure 14. EMA/Pfizer-BioNTech Comirnaty Assessment Report, 19 February 2021, page 23.

[7] OECD - Guidance Manual for the Testing of Manufactured Nanomaterials: OECD's Sponsorship Programme; ENV/JM/MONO(2009)20/REV first revision – 02 Jun 2010, pp. 58 https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2009)20/rev&doclanguage=en

provide, in the shortest possible time, a plausible and logical technical explanation of the occurrence of such a criticality? What were the remedies provided to avoid its recurrence?

These are valid questions, considering that, a few pages later, the author of the EMA report (2021, p.37), acknowledges that:

> Since *mRNA integrity and polydispersity are CQAs [8] for the efficacy of the medicinal product*, the finished product acceptance criteria for these parameters should be revised as further data becomes available from ongoing clinical trials and in line with manufacturing process capability. Due date: *July 2021*, Interim reports: *March 2021*. [my emphasis]

It is therefore presumable, although not confirmed, that the anomalous variations relating to the Polydispersity index of some batches were subsequently resolved and reported to EMA by July 2021.

What, then, were the *stabilized* PI mean values (that is not subject to "occasional" variability) of each specific batch examined? To which specific phase of the industrial process were the previously found "anomalies" in the polydispersity values related? Why did they happen in certain batches and not in others? What were the *root causes* that, once identified, provided an unequivocal technical and scientific explanation for the occurrence of such a criticality? What were the remedies adopted to avoid its recurrence? What, ultimately, were the *CA/PA* (*Corrective Actions/ Preventive Actions*) adopted in order to assure EMA (and, above all, the future patients subjected to inoculation) that such a critical phenomenon could never occur again?

Unfortunately, these questions, at the date of this paper, still await detailed and exhaustive answers. In the absence of sufficient information and official confirmations, we can, however, formulate some hypotheses, which, once verified by the appropriate clinical or medico-legal authorities, could provide further explanations and definitive confirmations both regarding the chemical-physical instability of the Comirnaty preparation and the consequent immunological and toxicological risks that such instability can cause and/or has already regrettably caused.

ZETA POTENTIAL AND INSTABILITY OF COLLOIDAL SYSTEMS

The Comirnaty medicinal preparation is, in essence, described, on a chemical-physical level, as:

> *A colloidal suspension, thermodynamically unstable, consisting of lipid nanoparticles and their aggregates or agglomerates, characterized by a variable Polydispersity index.*

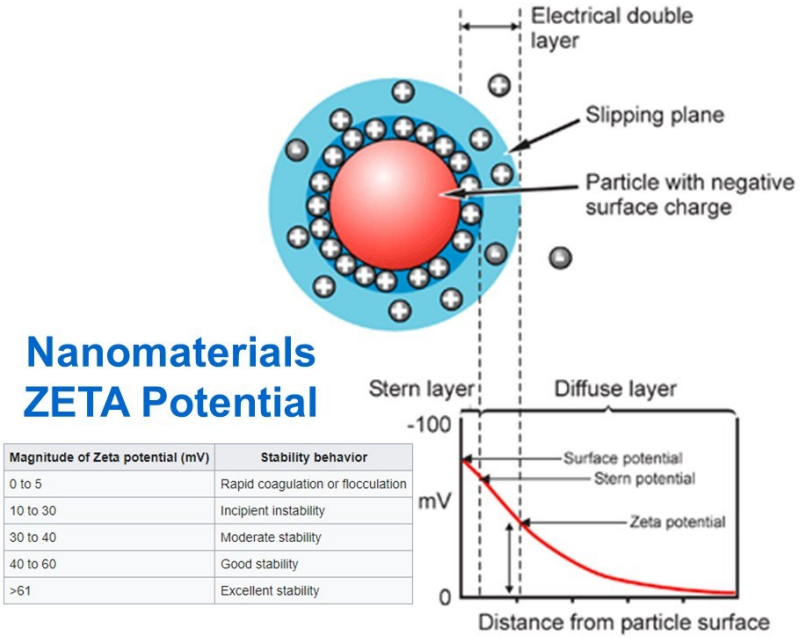
The term *colloid* derives from the Greek *kòlla,* glue, gluten, with the adjectival suffix *-oid*, which indicates *similarity*, *affinity*, that is, similar to glue: it therefore appears as an amorphous mass that, diluted in water, forms a more fluid colloidal dispersion (hence more suitable for parenteral administrations).

A *colloidal suspension* is simply a mixture in which dispersed solid particles (in this case *lipid* nanoparticles) remain suspended in an aqueous dispersing medium, for more or less long periods of time. A suspension of very small particles (such as those formed by Comirnaty lipids) can theoretically approach a real solution in appearance. In general, the system becomes more stable (durable over time) if the dispersed particles are smaller, if the densities of the two phases

[8] CQAs: Critical Quality Attributes

(dispersed and dispersing) are made nearer the same, and if the density of the dispersing phase is made greater (Stokes' law).

The propensity of particles to associate into aggregates or agglomerates (and therefore their polydispersity index) depends on another important parameter, which the manufacturer of nanoforms is required to measure, record, and report to regulatory authorities: the *Zeta potential*.



Magnitude of Zeta potential (mV)	Stability behavior
0 to 5	Rapid coagulation or flocculation
10 to 30	Incipient instability
30 to 40	Moderate stability
40 to 60	Good stability
>61	Excellent stability

Figure 15. The Zeta potential basically defines the stability of the colloidal suspension.

The Zeta potential, or electrokinetic potential (referred to the letter zeta "ζ" of the Greek alphabet) is the potential generated as a result of the formation of an electric double layer around the individual particles. (Figure 15). It represents the key factor for the determination of electrokinetic phenomena and stability of colloidal systems and, consequently, of the bioavailability of a compound or drug carried by nanoforms and intended to cross cellular or subcellular membranes (OECD, 2010, pp. 33, 63).

As described in an article (Barone et al., 2017) published by the Italian Agency *ENEA* (*Energia Nucleare Energie Alternative*, Agency for New Technologies, Energy and Sustainable Economic Development):

> A nanoparticle placed in solution forms a *colloidal system* for a longer or shorter time. A greater stability of the colloidal systems prevents the *phenomenon of aggregation of particles* as electrostatic repulsions originate that favor their dispersion. The parameter used to calculate colloidal stability is the *Zeta potential* which refers to the *potential generated by a double layer of electric charges*. *When the potential is low*, attractive forces prevail over repulsive and therefore *more aggregates will form*.
>
> The knowledge of the real concentration of the particles to which the biological system is exposed is important to determine the *estimation of the health risk* and can be expressed both as particle number and as total surface area and is *strongly affected by the degree of aggregation of the particles*. In in-vitro experiments, the variation of these parameters can affect the *greater or lesser degree of endocytosis (internalization of particles by cells)*, which is *important for defining the mode of action of that nanomaterial*. [my emphasis]

In summary, a high Zeta potential value (e.g. 40 to 60 mV) gives greater stability to colloidal systems, as electrostatic repulsions arise that prevent the aggregation of dispersed particles. When the potential is low (e.g. from 5 to 10 mV), attractive forces prevail over repulsive ones and therefore it is easier for processes such as *agglomeration*, or even *flocculation*, to occur (OECD, 2010, p. 63). The latter is nothing more than the formation of *coarse particulate matter*, sometimes, but not always, visible even to the naked eye. This is the stage that could lead to

coalescence, a phenomenon that occurs when the film surrounding the particles breaks and the aggregates of various sizes combine with each other to form a larger agglomeration (cluster), finally determining the "breaking" of the dispersion and the *separation of the phases* (Fig. 16, 17).

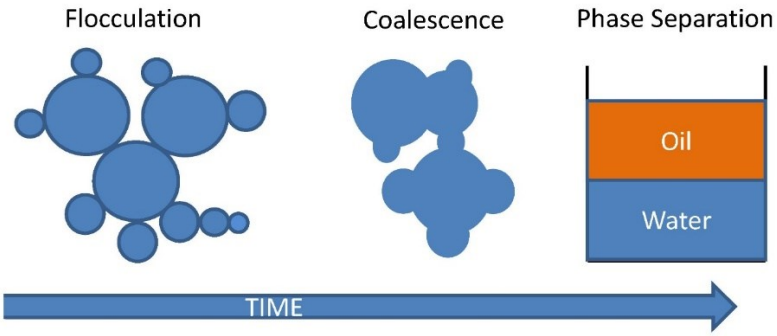


Figure 16. Flocculation, coalescence and phase separation in an unstable colloidal system.

CAUSES OF INSTABILITY OF COLLOIDAL SYSTEMS

The causes that lead to the instability of a colloidal biphasic system are many, and must always be analyzed and identified case-by-case with the appropriate laboratory instruments. Among the most common, for example, are: incorrect ratios between the dispersed phase and the dispersing phase; wrong method of processing; cooling or heating temperatures too high or too low; excessive air absorption that could change the ratios of the biphasic system; and, above all, the *presence of electrolytes* (Bushmanova et al., 1994).

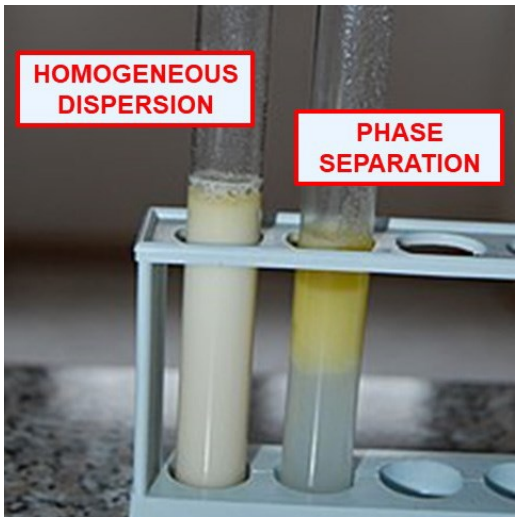


Figure 17. "Breaking" of homogeneous dispersion and separation of the two phases.

An electrolyte is a substance that in solution or in the molten state undergoes the division into ions (electrically charged particles) of its molecules. Substances that do not dissociate into electrically charged particles are called "non-electrolytes". The term "electrolyte" refers to the ability to conduct electric current thanks to the intervention of ions, a peculiar characteristic of these chemical species. Inorganic mineral salts, such as sodium chloride, are the most classic example (NaCl dissociates into Na^+ and Cl^- ions).

Electrolytes, depending on the concentrations involved, *can considerably alter the Zeta potential* of a colloidal dispersion, *causing the aggregation and agglomeration of nanoparticles*, and their subsequent *flocculation* by electrostatic attraction (Tadros, 2018). In other words: the *addition of electrolytes is one of the most common causes of the variation of the Zeta potential* and the *Polydispersity index* and, consequently, of the *instability of the colloid*, with all the easily predictable consequences that this entails, both with regard to the ineffectiveness and to the toxicological risks that will characterize the preparation itself, as already described above.

COMPOSITION OF THE MEDICINAL PRODUCT COMIRNATY

Comirnaty is originally supplied as a concentrated multi-dose liquid preparation (0.45 mL volume), stored frozen between -90°C and -60°C in a 2 mL glass vial, and to be diluted shortly before inoculation with a sodium chloride solution for injection.

The vial is thawed by keeping it in a refrigerator (2°C to 8°C) for 2 to 3 hours or at room temperature (up to 25°C) for 30 minutes. Once returned to room temperature, the multi-dose vial is diluted with 1.8 mL of the 9 mg/mL (0.9%) sodium chloride solution. After dilution, each vial of Comirnaty contains 2.25 mL from which 6 doses of 0.3 mL of vaccine can be extracted. Each dose contains 30 µg of the active ingredient (that is the mRNA BNT162b2, intended to code for the SARS-CoV-2 spike glycoprotein) and the excipients listed in Table 1. After dilution, according to the instructions of Pfizer-BioNTech, the vials are stored at a temperature between 2°C and 25°C and should be used within 6 hours (FDA, 2021, p.4).

Table 1. Composition of one dose of Comirnaty vaccine after addition of a physiological sodium chloride solution (*Electrolytes).

Ingredient	**Function**	**Quantity per dose**
BNT162b2	Active	30 µg
ALC-0315	Functional Lipid	0,43 mg
ALC-0159	Functional Lipid	0,05 mg
DSPC	Structural Lipid	0,09 mg
Cholesterol	Structural Lipid	0,2 mg
Sucrose	Cryoprotective	6 mg
Sodium chloride *	pH buffer & diluent solution component	2,52 mg
Potassium chloride*	pH buffer component	0,01 mg
Sodium phosphate dibasic dihydrate*	pH buffer component	0,07 mg
Potassium dihydrogen phosphate*	pH buffer component	0,01 mg
Water for injection	Dispersing medium	q.s. to 0.3 ml

PRESENCE OF ELECTROLYTES IN THE COMPOSITION OF THE COMIRNATY MEDICINAL PRODUCT

As shown in Table 1 and Figure 18, the formulation of the Pfizer-BioNTech COVID19 vaccine contains *4 electrolytes* (inorganic salts), components of the pH buffer, used to stabilize the pH of the preparation at a value between 6.9 and 7.9: sodium chloride, potassium chloride, sodium dibasic phosphate dihydrate, potassium dihydrogen phosphate.

Note that, in the final composition of Comirnaty, that is after dilution with a 0.9% sodium chloride solution, the quantitative proportion (by weight) between the total amount of electrolytes present and that of the two functional lipids is 5.44:1. In fact, for every dose of vaccine inoculated, we have *2.61 mg of electrolytes versus only 0.48 mg of ALC-0315 + ALC-0159*. A quantity that turns out to be

more than 5 times the amount of the two functional lipids responsible for the formation of nanoparticles in suspension. The ratio by weight of lipid ALC-0315 (cationic) to mRNA (anionic) is 14:1.

Summary Basis for Regulatory Action

Date:	11/8/2021
From:	Ramachandra Naik, PhD, Review Committee Chair, DVRPA/OVRR
BLA STN:	125742/0
Applicant:	BioNTech Manufacturing GmbH (in partnership with Pfizer, Inc.)

The COMIRNATY Multiple Dose Vial is thawed in a refrigerator (2°C to 8°C) for 2 to 3 hours or at room temperature (up to 25°C) for 30 minutes. The vial must be warmed to room temperature for dilution. Once at room temperature, the COMIRNATY Multiple Dose Vial is diluted with 1.8 mL of the diluent. After dilution, each vial of COMIRNATY contain six doses of 0.3 mL of vaccine. Each 0.3 mL dose of COMIRNATY contains 30 µg of mRNA encoding the spike glycoprotein of SARS-CoV-2 and the following ingredients: lipids (0.43 mg ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate), 0.05 mg 2-(polyethylene glycol 2000)-N,N-ditetradecylacetamide, 0.09 mg 1,2-distearoyl-sn-glycero-3-phosphocholine, and 0.2 mg cholesterol), 0.01 mg potassium chloride, 0.01 mg monobasic potassium phosphate, 2.52 mg sodium chloride, 0.07 mg dibasic sodium phosphate dihydrate, and 6 mg sucrose. After dilution, the vials are stored at 2°C to 25°C and must be used within 6 hours from the time of dilution. COMIRNATY is preservative-free.

Page 4

Figure 18. Pfizer-BioNTech COVID19 Vaccine — FDA Document at https://www.fda.gov/media/151733/download : Summary Basis for Regulatory Action - 11/08/2021.

The key question here is: can such a colloidal suspension be considered stable?

By diligently evaluating the above data regarding the Zeta potential and the Polydispersity Index, the answer can only be negative: such a high relative concentration of electrolytes, in such a precarious colloidal suspension, can only lead to a *drastic reduction of the Zeta potential*, with consequent predictable phenomena of *aggregation, agglomeration***,** and, finally, *flocculation***.**

Moreover, examining the EMA official document *Annex I Comirnaty Summary of Product Characteristics*, it is clear that both the manufacturer and the authorizing bodies were well aware of the risks relating to its instability and the obvious possibility of coarse particulate formation *in situ*, shortly before administration. In fact, the dilution instructions read:

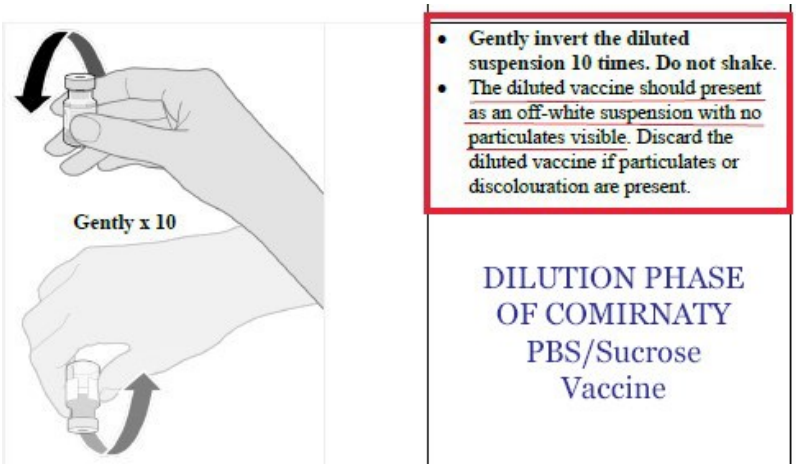


Figure 19. Dilution/mixing phase of Comirnaty lipid nanoparticles suspension.

- *Gently* invert the diluted dispersion *10 times. Do not shake.*

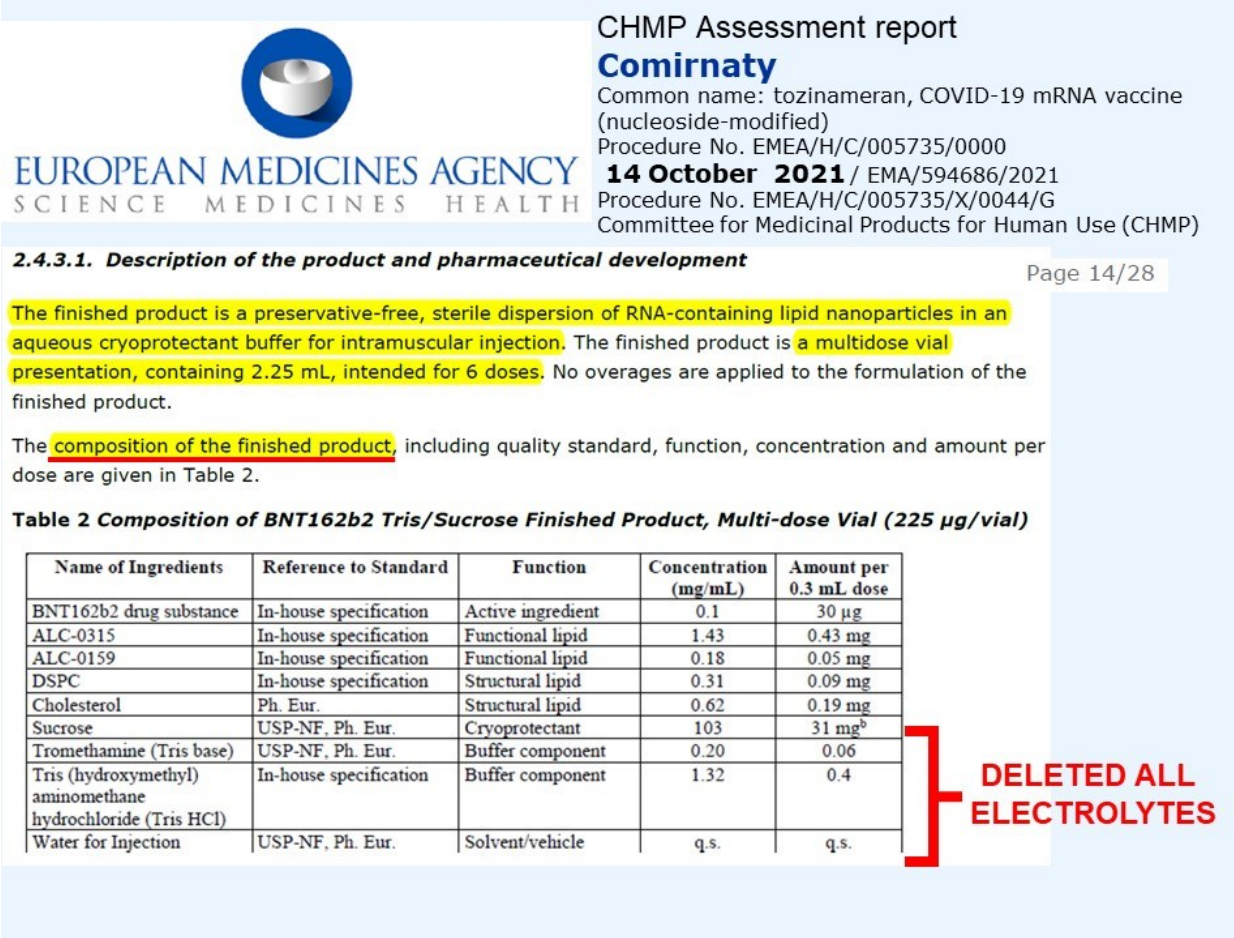
> • The diluted vaccine should present as an off-white suspension, with no particulates visible. *Discard the diluted vaccine if particulates or discolouration are present* [Figure 19].

Leaving aside the improper use, in the text intended for vaccinating operators, of the term *particulates*, rather than the more appropriate one of *flocculates*, we cannot avoid asking some important questions concerning the assessment of the specific risks related to the inspection of the vial, after the dilution phase:

- What is the value of the Zeta potential of nanoparticle suspension after dilution with the sodium chloride electrolytic solution?
- What is the difference between the value of the Zeta potential after dilution and that of the concentrated suspension (before dilution)?
- What is the value of the Zeta potential of the diluted suspension, under average physiological conditions (such as pH 7.4; 2.2 mM Ca^{++}) at 37°C, that is to say at the conditions to which it is subjected a few moments after intramuscular inoculation?
- How accurate should the inspection of the vial be, after dilution, in order to minimize errors (accidental and systematic) in assessing the presence or absence of aggregates or agglomerates or flocculates?
- How numerous and "visible" must the "particulates" be in order to trigger in the observer the decision-making act of discarding the non-compliant vial?
- How "gently" should the vial be turned upside down (that is to say *inverted*, but not *shaken*)?
- What values of Zeta potential are obtained in case of minimum or excessive shaking of the vial?
- What would be the possible risks related to an error (reasonably understandable and most likely motivated by fatigue or nervous tension or absent-mindedness by the vaccinating doctor) in the number of overturns of the vial? In other words, if the reversals, instead of 10, were 8, or 12, or 5, what would be the risk, in these cases, of obtaining an insufficient homogeneity (and therefore a greater instability) of the diluted suspension?
- Who verifies and controls the evaluator of the dilution/ inverting/ visual inspection procedures with regard to approval of compliant vials or rejection of non-compliant vials?
- How many vials, statistically, have been detected as non-compliant? Are the statistical findings significantly consistent among the various vaccination operators and the different vaccination sites?

These are, of course, just some of the most relevant questions that emerge from elementary but necessary evaluations and considerations related to fundamental parameters, such as the Polydispersity index and the Zeta potential, and, consequently, to the degree of stability of the resulting colloidal suspensions. However, these are questions that require accurate and prompt answers, taking in consideration, above all, the serious consequences that any error, omission or

negligence in the dilution phase could entail, from a statistical-epidemiological, clinical and medico-legal point of view, for the safety of those who undergo intramuscular inoculation of a liquid suspension of nanoparticles which could be excessively poly-dispersed, or even close to flocculation or coalescence or phase separation.



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

CHMP Assessment report
Comirnaty
Common name: tozinameran, COVID-19 mRNA vaccine (nucleoside-modified)
Procedure No. EMEA/H/C/005735/0000
14 October 2021 / EMA/594686/2021
Procedure No. EMEA/H/C/005735/X/0044/G
Committee for Medicinal Products for Human Use (CHMP)

2.4.3.1. Description of the product and pharmaceutical development

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The finished product is a preservative-free, sterile dispersion of RNA-containing lipid nanoparticles in an aqueous cryoprotectant buffer for intramuscular injection. The finished product is a multidose vial presentation, containing 2.25 mL, intended for 6 doses. No overages are applied to the formulation of the finished product.

The composition of the finished product, including quality standard, function, concentration and amount per dose are given in Table 2.

Table 2 *Composition of BNT162b2 Tris/Sucrose Finished Product, Multi-dose Vial (225 µg/vial)*

Name of Ingredients	Reference to Standard	Function	Concentration (mg/mL)	Amount per 0.3 mL dose
BNT162b2 drug substance	In-house specification	Active ingredient	0.1	30 µg
ALC-0315	In-house specification	Functional lipid	1.43	0.43 mg
ALC-0159	In-house specification	Functional lipid	0.18	0.05 mg
DSPC	In-house specification	Structural lipid	0.31	0.09 mg
Cholesterol	Ph. Eur.	Structural lipid	0.62	0.19 mg
Sucrose	USP-NF, Ph. Eur.	Cryoprotectant	103	31 mg[b]
Tromethamine (Tris base)	USP-NF, Ph. Eur.	Buffer component	0.20	0.06
Tris (hydroxymethyl) aminomethane hydrochloride (Tris HCl)	In-house specification	Buffer component	1.32	0.4
Water for Injection	USP-NF, Ph. Eur.	Solvent/vehicle	q.s.	q.s.

DELETED ALL ELECTROLYTES

Figure 20. New composition (called Tris/Sucrose Finished Product) of the Pfizer-BioNTech Comirnaty vaccine, electrolyte-free, ready to use, no longer requiring the dilution phase.

ELIMINATION OF ELECTROLYTES IN THE COMPOSITION OF THE NEW MEDICINAL PRODUCT COMIRNATY "TRIS"

On 2021, October 18, EMA announced, on its official website, that *EMA's human medicines committee (CHMP) has approved two additional manufacturing sites for the production of Comirnaty, the COVID-19 vaccine developed by BioNTech and Pfizer. One site, located in Monza, Italy, is operated by Patheon Italia S.P.A. The other in Anagni, also in Italy, is operated by Catalent Anagni S.R.L. Both sites will manufacture finished product. These sites will produce up to 85 million additional doses to supply the EU in 2021.*

And, surprisingly, on the same page, it also announced that:

> […] The CHMP has approved *a ready-to-use formulation of Comirnaty*. This formulation *does not require dilution prior to administration*, will be available in a 10-vial (60 dose) pack size and can be stored at 2-8°C for up to 10 weeks. The current concentrated formulation requires dilution prior to

> administration, is available in a 195-vial (1,170 dose) pack size and can be stored at 2-8°C for up to one month). These differences will provide *improved storage, transport and logistic options* for vaccine distribution and administration. The new formulation will be available in a phased rollout starting in early 2022. [my emphasis]

According to such peculiar announcement, the *new Comirnaty formulation, ready for inoculation, no longer requires dilution*, with obvious advantages of *storage, transport and logistics***.** It is assessed and authorized under the same conditions as the previous one, but in a new EMA Assessment report, dated 2021, October 14, entitled *CHMP assessment report on group of an extension of marketing authorisation and variations*, in accordance with Reg. (EC) No 1234/2008, and with the premise that it is an Assessment report as adopted by the CHMP *with all information of a commercially confidential nature deleted*.

And, on page 14 of such report, the new formulation is revealed (Figure 20), and, with it, some details that tend to confirm, both on the chemical-physical and toxicological level, the above detailed evaluation concerning the manifest instability and potential danger of the original Comirnaty flawed formulation.

Oddly enough, in the new composition of Comirnaty, called *Tris/Sucrose Finished Product,* containing the same active ingredient (mRNA chemically modified at nucleoside level), the same functional lipids and the same supporting excipients (at the same concentrations), all the electrolytes that were present in the previous *electrolytic* formulation (called, for the occasion, *PBS/Sucrose Finished Product*, where PBS stands for *Phosphate-Buffered Saline*), have totally disappeared, of course without providing the reader with any explanation.

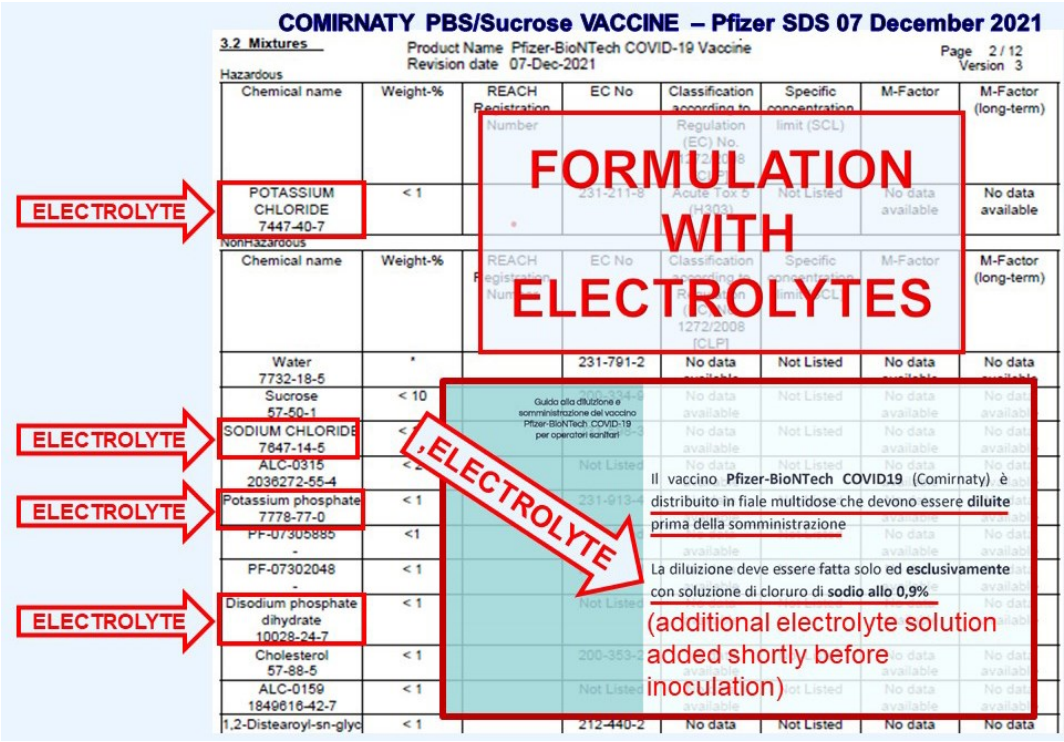
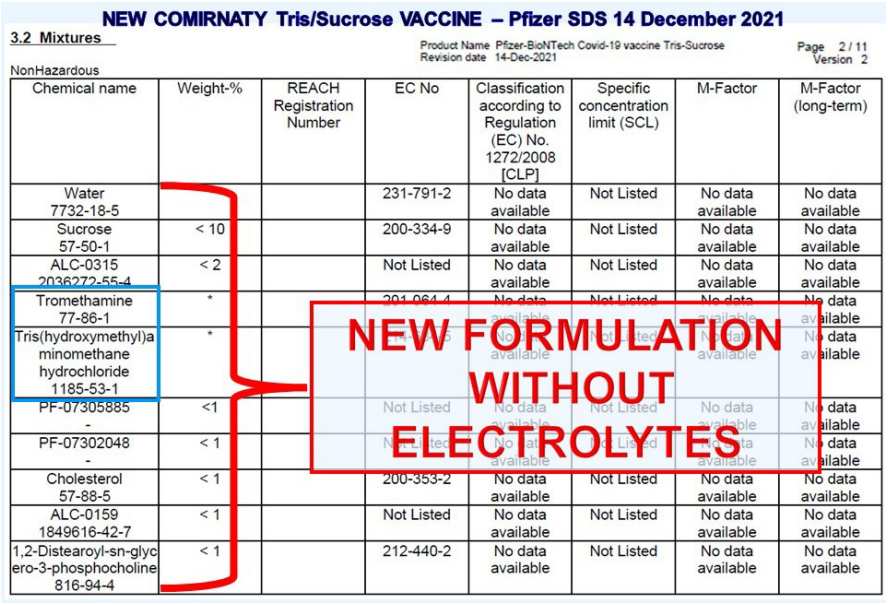


Figure 21.Pfizer-BioNTech Comirnaty PBS/Sucrose vaccine (Electrolytic) Safety Data Sheet section 3.2., 2021, December 7.

This *electrolyte purging* operation may seem ordinary to non-experts, but in reality it is revealing to experts in the field of colloidal systems, and even more explicit when comparing the relevant

sections 3.2. of the Safety Data Sheets of Comirnaty PBS/Sucrose (the *Electrolytic vaccine,* Fig. 21) and Comirnaty Tris/Sucrose (the *Non-electrolytic vaccine*) [Figure. 22].



NEW COMIRNATY Tris/Sucrose VACCINE – Pfizer SDS 14 December 2021

3.2 Mixtures

Product Name Pfizer-BioNTech Covid-19 vaccine Tris-Sucrose
Revision date 14-Dec-2021

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Version 2

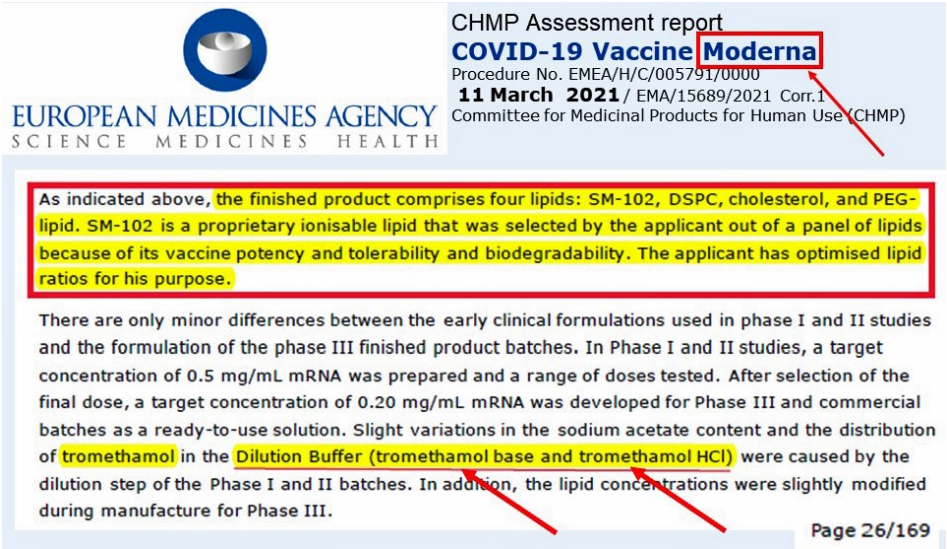
NonHazardous

Chemical name	Weight-%	REACH Registration Number	EC No	Classification according to Regulation (EC) No. 1272/2008 [CLP]	Specific concentration limit (SCL)	M-Factor	M-Factor (long-term)
Water 7732-18-5			231-791-2	No data available	Not Listed	No data available	No data available
Sucrose 57-50-1	< 10		200-334-9	No data available	Not Listed	No data available	No data available
ALC-0315 2036272-55-4	< 2		Not Listed	No data available	Not Listed	No data available	No data available
Tromethamine 77-86-1	*		201-064-4	No data available	Not Listed	No data available	No data available
Tris(hydroxymethyl)aminomethane hydrochloride 1185-53-1	*		[illegible]	No data available	Not Listed	No data available	No data available
PF-07305885 -	<1		Not Listed	No data available	Not Listed	No data available	No data available
PF-07302048 -	< 1		Not Listed	No data available	Not Listed	No data available	No data available
Cholesterol 57-88-5	< 1		200-353-2	No data available	Not Listed	No data available	No data available
ALC-0159 1849616-42-7	< 1		Not Listed	No data available	Not Listed	No data available	No data available
1,2-Distearoyl-sn-glycero-3-phosphocholine 816-94-4	< 1		212-440-2	No data available	Not Listed	No data available	No data available

NEW FORMULATION WITHOUT ELECTROLYTES

Figure 22. Pfizer-BioNTech Comirnaty TRIS/Sucrose vaccine (NON-electrolytic) Safety Data Sheet section 3.2., 2021, December 14.

From a technical point of view, in the new *ready to use* formulation, the previous pH buffer *PBS* (electrolytic, inorganic) has been eliminated and replaced with another very common buffer called *Tris buffer*, widely used in biology to prepare pH-controlled solutions (especially for nucleic acids), in



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

CHMP Assessment report
COVID-19 Vaccine Moderna
Procedure No. EMEA/H/C/005791/0000
11 March 2021 / EMA/15689/2021 Corr.1
Committee for Medicinal Products for Human Use (CHMP)

As indicated above, the finished product comprises four lipids: SM-102, DSPC, cholesterol, and PEG-lipid. SM-102 is a proprietary ionisable lipid that was selected by the applicant out of a panel of lipids because of its vaccine potency and tolerability and biodegradability. The applicant has optimised lipid ratios for his purpose.

There are only minor differences between the early clinical formulations used in phase I and II studies and the formulation of the phase III finished product batches. In Phase I and II studies, a target concentration of 0.5 mg/mL mRNA was prepared and a range of doses tested. After selection of the final dose, a target concentration of 0.20 mg/mL mRNA was developed for Phase III and commercial batches as a ready-to-use solution. Slight variations in the sodium acetate content and the distribution of tromethamol in the Dilution Buffer (tromethamol base and tromethamol HCl) were caused by the dilution step of the Phase I and II batches. In addition, the lipid concentrations were slightly modified during manufacture for Phase III.

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Figure 23. EMA Assessment Report, 2021, March 11, p. 26. Composition of Moderna's *COVID*-19 Vaccine Spikevax.

pH ranges between 7 and 9. This is an organic buffer (meaning it does NOT contain inorganic electrolytes), which stabilizes the pH of the Comirnaty Tris/Sucrose product at a physiological value

of 7.4 [9], chemically consisting of *tris(hydroxymethyl) aminomethane (*also known as *tromethamine* or *tromethamol*) and its hydrochloride. It is interesting to note that, eliminating the electrolyte buffer and substituting the new organic buffer based on trometamol, the entire formulation of the new Pfizer-BioNTech preparation called *Tris Sucrose* becomes, if not identical, at least very similar to that of *Moderna's Spikevax vaccine* (the latter authorized by EMA on 2021, January 6, Assessment report 2021, March 11). In fact, both of these vaccines include the following elements: a nucleoside-modified mRNA + a cationic functional lipid + a polyethoxylated lipid + a neutral lipid (DSPC) + cholesterol + the *non-electrolytic tromethamol-based pH buffer* (Figure 23).

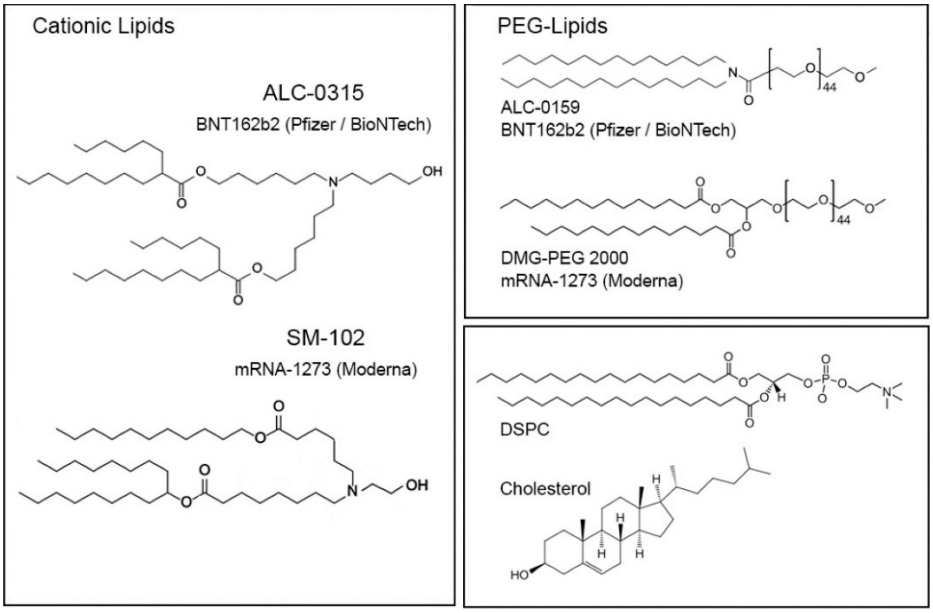


Figure 24. Comparison between lipids used in the Pfizer-BioNTech mRNA vaccine and Moderna's mRNA vaccine (Tenchov et al., 2021).

As stated by the EMA itself, on page 22 of its Assessment report dated 14 October 2021 concerning the new Comirnaty Tris/Sucrose:

> [...] *the Tris/Sucrose finished product* has been developed to provide a vaccine with *an improved stability profile* and *greater ease of use at administration sites*. [my emphasis]

How much has the *stability profile* been *improved*, compared to that of the previous version, is unfortunately not revealed. In other words, the key question here is: what is the variation between the Polydispersity index of the non-electrolytic Tris/Sucrose vaccine and that of the electrolytic PBS/Sucrose vaccine? What is the variation between the Zeta potential of the lipid nanoparticles of the ready-to-use non-electrolytic Tris/Sucrose vaccine and that of the electrolytic PBS/Sucrose vaccine diluted and gently inverted 10 times before inoculation? What is the Zeta potential of the new Tris/Sucrose formulation, under physiological conditions (i.e., pH 7.4; 2.2 mM Ca^{++}) and at the

[9] In the absence of pathological states, the pH of the human body ranges between 7.35 to 7.45, with the average at 7.40 (Hopkins et al. 2022).

temperature of 37°C, that is the temperature to which it is subjected a few instants after intramuscular injection?

Unfortunately, the answers to these questions, may never arrive, since they are likely to be judged as pertaining to *information of a confidential commercial nature* and therefore subject to suppression. But, nevertheless, the logical observation that *the Comirnaty PBS/Sucrose vaccine* (injected in billions of doses) *had to be modified*, not only for greater ease of use, but also, first and foremost, *to improve its stability,* remains and will always remain insuppressible, as written in an official EMA report. And this will remain, among other things, the first simple admission, albeit indirect and swamped by "logistic" justifications, that the previous PBS/Sucrose electrolytic version**,** *being NOT sufficiently stable*, consequently *presented greater toxicological risks* and subsequently *had to be corrected* by Pfizer-BioNTech and *grossly transmuted* into a new version promptly authorized by EMA.

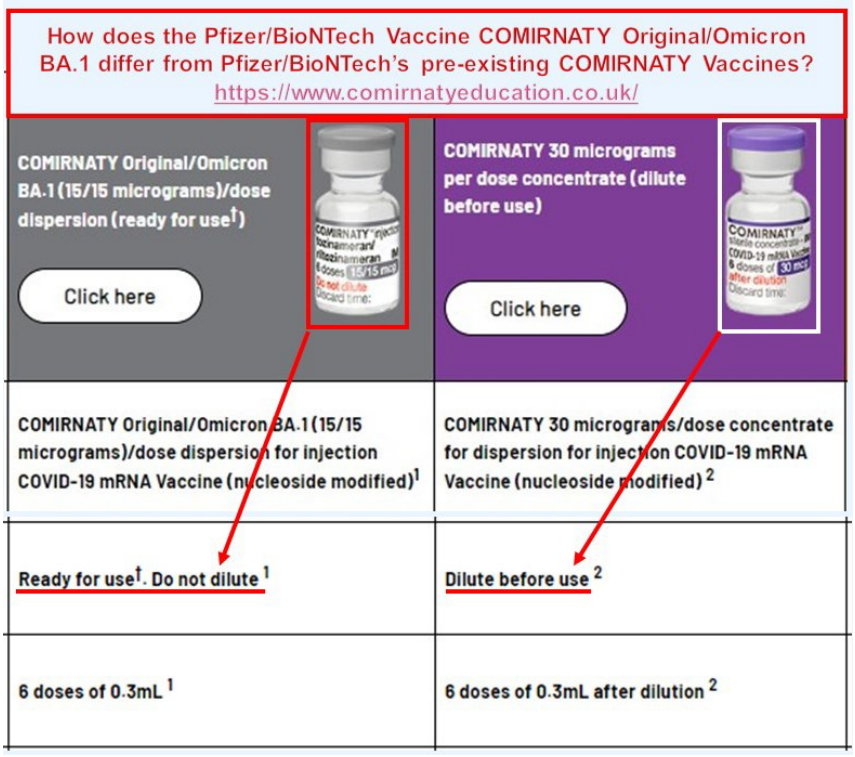


Figure 25. The two co-existing Pfizer/BioNTech Vaccines.

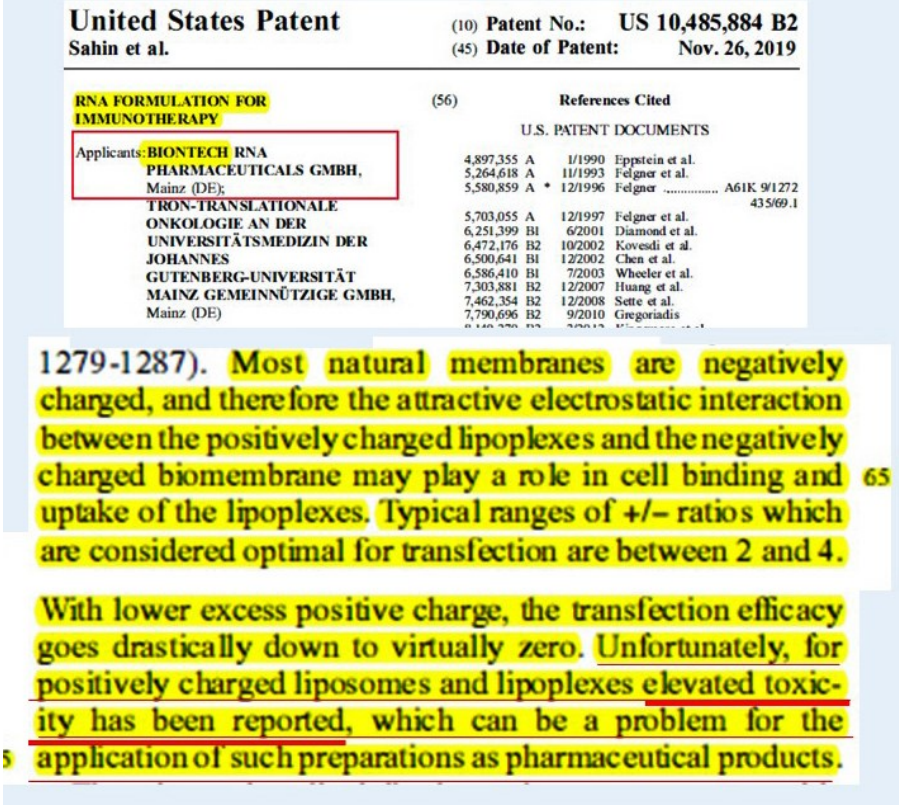
However, what seems very odd is that the new non-electrolytic version Tris/Sucrose is presented on the web only as a mere pharmaco-technological development (a simple *upgrade*) as well as an admirable solution to the onerous problems of *storage, transport, and logistics*, without making any mention of the toxicological risks and dangers to public health that the previous formulation implied. In fact, the old electrolytic version, though unstable and to be diluted before inoculation, remains surprisingly on the market, simply distinguished by a *purple cap* (dilution necessary, for subjects aged 12 years and older), next to the new version with *gray cap* (dilution NOT necessary, also for subjects aged 12 years or older). Both versions are in fact authorized by EMA for placing on the market in Europe, always with the formula *authorization subject to conditions* and considered equivalent and interchangeable with each other (Figure 25).

INSTABILITY AND TOXICOLOGICAL POTENTIAL OF THE *COMIRNATY PBS/SUCROSE* VERSION: CONFIRMATIONS AND CROSSCHECKS

In the light of the technical data set out above, it is now quite clear that the instability of the colloidal system of lipid nanomaterials (and their consequent greater toxicological risk) of the first version of Comirnaty is substantially due to the presence, in that formulation, of *destabilizing factors*, such as, in fact, the excess electrolytic inorganic compounds, which make up the *PBS pH-buffer*

therein used by Pfizer-BioNTech. In this regard, a clarifying definition of the concept of "stability", in relation to nanoparticles-based compositions, is reported in Moderna's patent US 10,442,756 B2 *Compounds and compositions for intracellular delivery of therapeutic agents*:

> *Stability*, *stabilized* and *stable* in the context of the present disclosure refers to the *resistance of nanoparticle compositions and/or pharmaceutical compositions disclosed herein to chemical or physical changes* (e.g., *degradation, particle size change, aggregation*, change in encapsulation, etc.) under given *manufacturing, preparation, transportation, storage* and/or *in-use conditions*, e.g., when stress is applied such as shear force, *freeze/thaw stress*, etc. [my emphasis]



United States Patent
Sahin et al.

(10) **Patent No.: US 10,485,884 B2**
(45) **Date of Patent: Nov. 26, 2019**

RNA FORMULATION FOR IMMUNOTHERAPY

Applicants: **BIONTECH RNA PHARMACEUTICALS GMBH**, Mainz (DE); **TRON-TRANSLATIONALE ONKOLOGIE AN DER UNIVERSITÄTSMEDIZIN DER JOHANNES GUTENBERG-UNIVERSITÄT MAINZ GEMEINNÜTZIGE GMBH**, Mainz (DE)

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1279-1287). Most natural membranes are negatively charged, and therefore the attractive electrostatic interaction between the positively charged lipoplexes and the negatively charged biomembrane may play a role in cell binding and uptake of the lipoplexes. Typical ranges of +/– ratios which are considered optimal for transfection are between 2 and 4.

With lower excess positive charge, the transfection efficacy goes drastically down to virtually zero. Unfortunately, for positively charged liposomes and lipoplexes elevated toxicity has been reported, which can be a problem for the application of such preparations as pharmaceutical products.

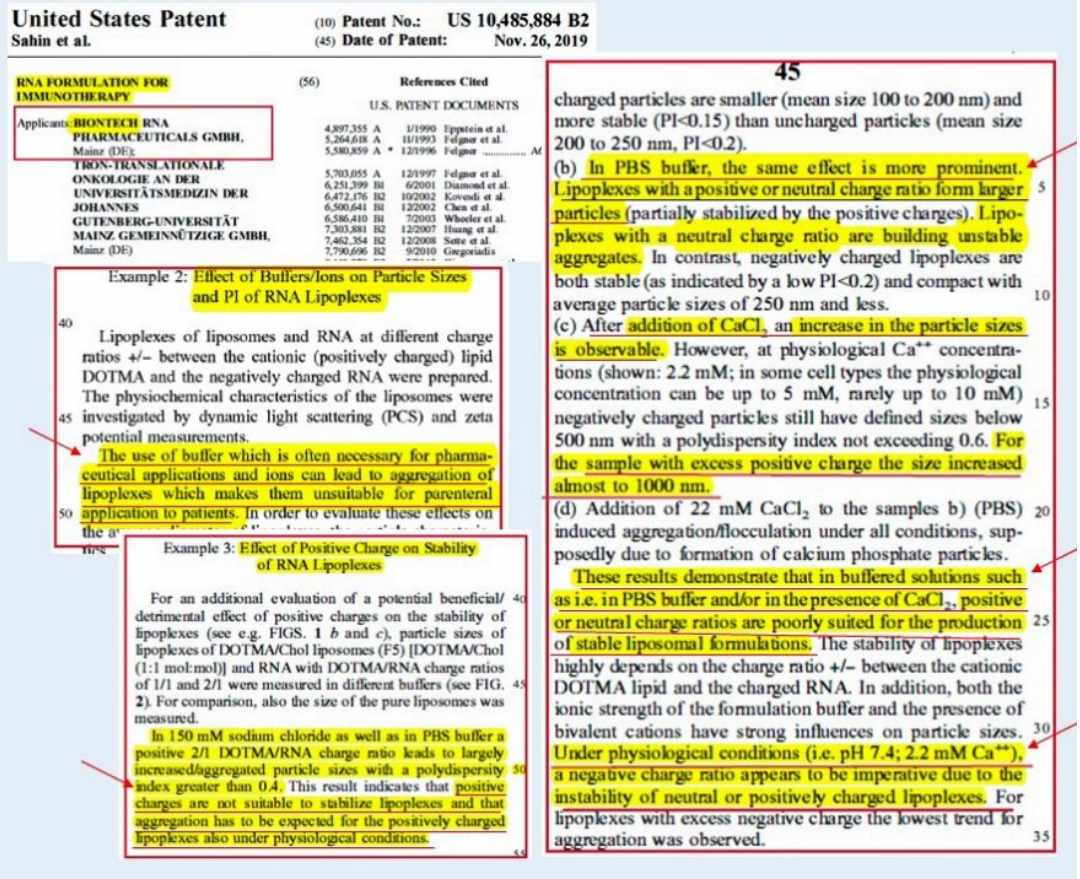
Figure 26. BioNTech Patent US 10,485,884 B2 RNA Formulation for Immunotherapy - Nov 26, 2019; section Background of the invention, 1 (62-67), 2 (1-5).

In this same regard, however, what is reported in the patent of the same *BioNTech* (co-owner, together with Pfizer, of the Comirnaty vaccine) US 10,485,884 B2 *RNA Formulation for Immunotherapy* dated November 26, 2019 is even more explicit concerning "elevated toxicity" attributed to "positively charged liposomes and lipoplexes". The reference is to formulations of RNA encapsulated in cationic lipid nanoparticles — of the same category as those used in Comirnaty — and called, in this context, "lipoplexes". In the description of the patent, it is explained, among other things, how cationic nanoparticles containing RNA are formed mainly thanks to certain mass/charge ratios between cationic (+) lipids and anionic (-) components of RNA, and how these ratios play a fundamental role also with regard to the passage of RNA-containing nanoparticles through the cell membrane and the consequent transfer of RNA inside the cell (*transfection*) to modify its functional characteristics:

> *Most natural membranes are negatively charged, and therefore the attractive electrostatic interaction between the positively charged lipoplexes and the negatively charged biomembrane may play a role in cell binding and uptake of the lipoplexes*. Typical ranges of+/- ratios which are considered optimal for transfection are between 2 and 4. With lower excess positive charge, the transfection efficacy goes drastically down to virtually zero. *Unfortunately, for positively charged liposomes and lipoplexes elevated toxicity has been reported, which can be a problem for the application of such preparations as pharmaceutical products*. [my emphasis] (Figure 26).

The reasons why pH-buffers of the PBS-type are absolutely not suitable in preparations based on RNA-incorporating cationic nanoparticles are explained very clearly in the section *Examples*, *Effects of Buffers/Ions on Particle Sizes and Polydispersity Index of RNA Lipoplexes* of the aforementioned BioNTech patent; US 10,485,884 B2, 44 (47-50), 45 (4-6), 45 (31-33):

> *The use of buffer* which is often necessary for pharmaceutical applications *and ions can lead to aggregation of lipoplexes which makes them unsuitable for parenteral application to patients* […]
>
> In PBS buffer, the same effect is more prominent. Lipoplexes with a positive or neutral charge ratio form larger particles (partially stabilized by the positive charges […]
>
> *Under physiological conditions* (i.e. pH 7.4; 2.2 mM Ca^{++}), *a negative charge ratio appears to be imperative* due to the *instability of neutral or positively charged lipoplexes*. [my emphasis] (Figure 27).



United States Patent
Sahin et al.
(10) Patent No.: US 10,485,884 B2
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RNA FORMULATION FOR IMMUNOTHERAPY

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(56) References Cited
U.S. PATENT DOCUMENTS

Example 2: Effect of Buffers/Ions on Particle Sizes and PI of RNA Lipoplexes

Lipoplexes of liposomes and RNA at different charge ratios +/– between the cationic (positively charged) lipid DOTMA and the negatively charged RNA were prepared. The physiochemical characteristics of the liposomes were investigated by dynamic light scattering (PCS) and zeta potential measurements.

The use of buffer which is often necessary for pharmaceutical applications and ions can lead to aggregation of lipoplexes which makes them unsuitable for parenteral application to patients. In order to evaluate these effects on the a

Example 3: Effect of Positive Charge on Stability of RNA Lipoplexes

For an additional evaluation of a potential beneficial/detrimental effect of positive charges on the stability of lipoplexes (see e.g. FIGS. 1 *b* and *c*), particle sizes of lipoplexes of DOTMA/Chol liposomes (F5) [DOTMA/Chol (1:1 mol:mol)] and RNA with DOTMA/RNA charge ratios of 1/1 and 2/1 were measured in different buffers (see FIG. 2). For comparison, also the size of the pure liposomes was measured.

In 150 mM sodium chloride as well as in PBS buffer a positive 2/1 DOTMA/RNA charge ratio leads to largely increased/aggregated particle sizes with a polydispersity index greater than 0.4. This result indicates that positive charges are not suitable to stabilize lipoplexes and that aggregation has to be expected for the positively charged lipoplexes also under physiological conditions.

45

charged particles are smaller (mean size 100 to 200 nm) and more stable (PI<0.15) than uncharged particles (mean size 200 to 250 nm, PI<0.2).

(b) In PBS buffer, the same effect is more prominent. Lipoplexes with a positive or neutral charge ratio form larger particles (partially stabilized by the positive charges). Lipoplexes with a neutral charge ratio are building unstable aggregates. In contrast, negatively charged lipoplexes are both stable (as indicated by a low PI<0.2) and compact with average particle sizes of 250 nm and less.

(c) After addition of $CaCl_2$ an increase in the particle sizes is observable. However, at physiological Ca^{++} concentrations (shown: 2.2 mM; in some cell types the physiological concentration can be up to 5 mM, rarely up to 10 mM) negatively charged particles still have defined sizes below 500 nm with a polydispersity index not exceeding 0.6. For the sample with excess positive charge the size increased almost to 1000 nm.

(d) Addition of 22 mM $CaCl_2$ to the samples b) (PBS) induced aggregation/flocculation under all conditions, supposedly due to formation of calcium phosphate particles.

These results demonstrate that in buffered solutions such as i.e. in PBS buffer and/or in the presence of $CaCl_2$, positive or neutral charge ratios are poorly suited for the production of stable liposomal formulations. The stability of lipoplexes highly depends on the charge ratio +/– between the cationic DOTMA lipid and the charged RNA. In addition, both the ionic strength of the formulation buffer and the presence of bivalent cations have strong influences on particle sizes. Under physiological conditions (i.e. pH 7.4; 2.2 mM Ca^{++}), a negative charge ratio appears to be imperative due to the instability of neutral or positively charged lipoplexes. For lipoplexes with excess negative charge the lowest trend for aggregation was observed.

Figure 27. Figure 27. BioNTech Patent US 10,485,884 B2 RNA Formulation for Immunotherapy - Nov. 26, 2019 (section Examples).

In other words, based on what is scientifically documented and reported in a *patent of the same BioNTech*, additionally to what already described concerning the intrinsic dangerousness of positively

charged lipid nanoparticles, we learn that *a colloidal system of cationic lipid nanoparticles incorporating mRNA*

1. *should NOT contain an ionic buffer such as PBS*, in order to prevent aggregation, agglomeration, flocculation of lipid nanoparticles, and all the toxicological consequences described above.
2. *should NOT contain ionic compounds* (such as sodium chloride), in order to prevent aggregation, agglomeration, flocculation of lipid nanoparticles, and all the toxicological consequences described above.
3. *should NOT be injected intramuscularly,* due to its instability when placed in the physiological environment of the extracellular district (pH 7.4; 2.2 mM Ca^{++}).

All three of these rigorous recommendations, reported in the aforementioned BioNTech patent of 2019, are shamelessly contradicted, or ignored, in 2020, both by Pfizer-BioNTech and by the certifying bodies, both on the nature of the Comirnaty formulation (ionic/electrolytic) and on its intended use (intramuscular injection).

In the final analysis, the medicinal preparation Comirnaty/PBS Sucrose from Pfizer-BioNTech, authorized by EMA in 2020, *presents serious and evident liabilities* on the chemical-physical and consequently toxicological level — liabilities, in open contrast with the specific and pertinent recommendations asserted by BioNTech itself in its aforementioned patent (US 10,485,884 B2).

On the basis of these confirmations and cross-checks, we can therefore hypothesize that the addition of such an important share of electrolytic compounds to the already precarious equilibrium of a colloidal system made of cationic nanoparticles, easily influenced by ionic charges, has inevitably conditioned the stability, shelf life, functionality, and consequent toxicological potential of the finished product Comirnaty PBS/Sucrose, causing in particular: unpredictable alterations of its Polydispersity index and Zeta potential; possible consequent formation of aggregates, agglomerates, flocculates, coalescences; different degrees of penetrability and mobility of nanolipid aggregates of different sizes, after inoculation, in unexpected and unpredictable biological sites, with irregular ROS formation at these sites; consequent heterogeneity of adverse effects (*randomization*), potentially variable from batch to batch, from vial to vial, from vaccinator to vaccinator, from vaccinated to vaccinated, in a sort of ineluctable, uncontrollable, and indecipherable Russian roulette (Santiago, 2022).

CONCLUSIONS

The *Comirnaty COVID-19 mRNA BNT162b2* vaccine, in its original version and composition, called *PBS/Sucrose*, presents numerous critical issues and drawbacks, examined in detail in this study and summarized as follows:

- The two functional excipients responsible for the formation of lipid nanoparticles, ALC-0315 and ALC-0159, are not registered in any Pharmacopoeia, nor are they among the substances examined and classified in accordance with Regulation (EC) No 1272/2008 on classification, labelling, and packaging of substances and mixtures in Europe (CLP).
- These excipients also do not appear in the inventory of substances registered in accordance with Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation, and Restriction of Chemicals in Europe (REACH). Therefore, their toxicological profile is not known in the first place.

- Not all the chemical-physical analysis procedures and toxicological tests required for nanoforms of these substances have been carried out, contrary to Regulation (EU) 2018/1881 amending Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation, and Restriction of Chemicals (REACH), to include the nanoforms of substances.
- Carcinogenicity, genotoxicity and mutagenicity studies of the preparation have not been carried out with the consent of the certifying body, although it has now been confirmed by numerous scientific studies that *Reactive Oxygen Species* (ROS), generated by nanoparticles, can have a high carcinogenic, genotoxic, and mutagenic potential.
- The Safety Data Sheets of the Comirnaty preparation do not report information on the characteristics of the nanoforms present in the composition of the preparation itself, contrary to the provisions of the aforementioned Regulation (EU) 2018/1881 and Regulation (EU) 2020/878.
- The actual values of the Polydispersity index and the Zeta potential of the nanoparticles present in the preparation are unknown. This leads to absolute uncertainty in the determination of the chemical-physical stability of nanoparticles and their aggregates, with consequent unpredictability inherent both to the potential efficacy of the vaccine and to the degree of penetrability and mobility of its nanoparticles within the human body, as well as their possible entry into the systemic circulation and accumulation in organs such as kidneys, liver, heart, brain, lungs.
- The presence of electrolytes in the original preparation (meaningfully eliminated in the subsequent *Comirnaty Tris/Sucrose* version) leads even more to the presumption that the product called *Comirnaty PBS/Sucrose* may give rise to the formation of aggregates and agglomerates before, during or after the inoculation procedure, and that it may therefore be both ineffective (since not able to convey the mRNA encoding the viral Spike protein of SARS-CoV-2 through the membranes of the host cell) and dangerous, as it would be deposited in tissues or organs not foreseen in its primary biological fate.

In conclusion, it is considered urgent and indispensable that an accurate and long-term study be carried out in the appropriate institutional, clinical or medico-legal seats, especially in relation to any causal or con-causal links between what is presented here and the wide pathological heterogeneity of serious or lethal adverse events that have occurred, or are occurring, after vaccinations, in order to adopt and expedite all appropriate corrective and preventive actions to protect public health, including discontinuing vaccinations with Pfizer-BioNTech Comirnaty PBS/Sucrose as soon as possible, in accordance with the precautionary principle, and in the light of 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.*

Funding and conflicts of interest

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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