

**Il Vaccino a mRNA:**  
**IL LATI OSCURI**

**Gabriele Segalla**

**nature reviews** drug discovery

**«Abbiamo pilotato l'aereo mentre lo stavamo ancora costruendo»**



**Kathrin Jansen**

AN AUDIENCE WITH | 11 November 2022

# **COVID vaccines: “We flew the aeroplane while we were still building it”**

**Recently retired head of vaccine R&D at Pfizer, Kathrin Jansen, discusses the lightning speed development of SARS-CoV-2 vaccines – and the implications for vaccine platforms.**

## COVID vaccines: “We flew the aeroplane while we were still building it”

<https://www.byoblu.com/2022/11/17/vaccini-pfizer-rivela-abbiamo-pilotato-laereo-mentre-lo-stavamo-costruendo/>

Tanti soldi, pochi dati e vari rischi. Le rivelazioni di Jansen

Jansen rivela alcuni particolari interessanti sulla messa a punto dei sieri a partire dai **finanziamenti**, che a quanto pare sono stati centrali: “Quando il CEO di Pfizer ha detto: ‘Fallo entro la fine dell’anno’ – racconta a Nature la ricercatrice -, ho risposto: ‘È una follia!’ Ma **il denaro non era un problema**, quindi, si potevano fare cose straordinarie in pochissimo tempo”. Nella ricostruzione della microbiologa si ammette un certo **margin**e di rischio a cui la popolazione è stata esposta: “Siamo diventati creativi: **non potevamo aspettare i dati, dovevamo fare così tanto a rischio**” afferma, e poi continua con una metafora: “**Abbiamo pilotato l’aereo mentre lo stavamo ancora costruendo**”. Jansen spiega che tutta la burocrazia è caduta, e conferma: “Stavamo facendo le cose **in parallelo**, guardando i dati e sviluppando la produzione. Di solito, la produzione non viene coinvolta se non **dopo anni** dall’inizio di un programma”. Nelle parole della ricercatrice emerge quanto la campagna vaccinale sia stata, alla fine, una **scommessa**: “Il Covid ha cambiato tutto in termini di come affrontare il concetto di **ricerca e sviluppo** di vaccini” rivela, aggiungendo che “**la piattaforma mRNA non era pronta per il lancio**. C’erano **problemi di stabilità, problemi di formulazione**, che dovevamo risolvere. Nel 2020, era solo un processo **di ricerca** e doveva essere ampliato”. Insomma la fretta e l’urgenza secondo la microbiologa di Pfizer hanno stravolto quello che è il normale iter per la messa a punto di un farmaco con una biotecnologia nuova.



**«CRITICITÀ CHIMICO-FISICHE E POTENZIALE  
TOSSICOLOGICO DEI NANOMATERIALI  
LIPIDICI CONTENUTI IN UN VACCINO COVID-19  
A mRNA»**

**IJVT  
PR**

**Chemical-physical criticality and toxicological potential of lipid nanomaterials contained in a COVID-19 mRNA vaccine**

**Gabriele Segalla**

Multichem R&D

Published

2023-01-26

ORCID: <https://orcid.org/0000-0002-5969-3732>

**DOI:** <https://doi.org/10.56098/ijvtpr.v3i1.68>



1. **INSTABILE**
2. **INSICURO**
3. **INEFFICACE**

**MEDICINALE IMPERFETTO**  
***(Art. 443 C.P.)***

**Chemical-physical criticality and toxicological potential of lipid nanomaterials contained in a COVID-19 mRNA vaccine**

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# MAGGIORI CRITICITÀ

- 1. Elevata penetrabilità, mobilità, reattività chimica e accumulazione sistemica delle nanoparticelle e dei loro aggregati in siti biologici imprevedibili.**
- 2. Elevata citotossicità dei lipidi ionizzabili e delle nanoparticelle lipidiche aventi carica positiva.**
- 3. Presenza di fattori destabilizzanti (es. elettroliti, diluizione, scongelamento, sbalzi termici).**



# Vaccino Pfizer/BioNTech «Comirnaty Covid-19 mRNA versione PBS/Sucrose»

## COMPOSIZIONE QUALI-QUANTITATIVA

|                  | Ingrediente                   | Funzione                           | Quantità per dose |
|------------------|-------------------------------|------------------------------------|-------------------|
| mRNA             | BNT162b2                      | Attivo                             | 30 µg             |
| Lipidi           | ALC-0315                      | Lipide funzionale                  | 0,43 mg           |
|                  | ALC-0159                      | Lipide funzionale                  | 0,0534 mg         |
|                  | DSPC                          | Lipide strutturale                 | 0,09 mg           |
|                  | Colesterolo                   | Lipide strutturale                 | 0,2 mg            |
|                  | Saccarosio                    | Crioprotettivo                     | 6 mg              |
| Tampone pH (PBS) | Cloruro di sodio              | Elettrolita: componente tampone pH | 2,52 mg           |
|                  | Cloruro di potassio           | Elettrolita: componente tampone pH | 0,01 mg           |
|                  | Fosfato disodico diidrato     | Elettrolita: componente tampone pH | 0,07 mg           |
|                  | Diidrogenofosfato di potassio | Elettrolita: componente tampone pH | 0,01 mg           |
|                  | Acqua per inoculazioni        | Mezzo disperdente                  | q.s. a 0,3 ml     |

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|                  | DSPC                          | Lipide strutturale                 | 0,09 mg           |
|                  | Colesterolo                   | Lipide strutturale                 | 0,2 mg            |
|                  | Saccarosio                    | Chlorofilativo                     | 6 mg              |
| Tampone pH (PBS) | Cloruro di sodio              | Elettrolita: componente tampone pH | 2,52 mg           |
|                  | Cloruro di potassio           | Elettrolita: componente tampone pH | 0,01 mg           |
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|                  | Acqua per inoculazioni        | Mezzo disperdente                  | q.s. a 0,3 ml     |

**Mai registrati in Farmacopea**

**Elettroliti (sali inorganici)**

## RNA FORMULATION FOR IMMUNOTHERAPY

Applicants: **BIONTECH RNA PHARMACEUTICALS GMBH**  
Mainz (DE);  
**FRONZ STRANZ GMBH**  
**ONKOLOGIE AN DER UNIVERSITÄTSMEDIZIN DER JOHANNES GUTENBERG-UNIVERSITÄT**

(56) References Cited

U.S. PATENT DOCUMENTS

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|-----------|-----|---------|----------------|
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| 5,264,618 | A   | 11/1993 | Felgner        |
| 5,580,859 | A * | 12/1996 | Felgner        |
| 5,703,055 | A   | 12/1997 | Felgner        |
| 6,251,399 | B1  | 6/2001  | Diamant        |
| 6,472,176 | B2  | 10/2002 | Koves          |
| 6,500,641 | B1  | 12/2002 | Chen et al.    |
| 6,586,410 | B1  | 7/2003  | Wheeler et al. |
| 7,203,981 | B2  | 12/2007 | Huang et al.   |

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

40 Lipoplexes of liposomes and RNA at different charge ratios +/- between the cationic (positively charged) lipid DOTMA and the negatively charged RNA were prepared. The physicochemical characteristics of the liposomes were  
45 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  
50 application to patients. In order to evaluate these effects on

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

For an additional evaluation of a potential beneficial/  
40 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  
45 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  
5 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<sub>2</sub>, an increase in the particle sizes  
10 is observable. However, at physiological Ca<sup>2+</sup> concentrations (shown: 2.2 mM; in some cell types the physiological concentration can be up to 5 mM, rarely up to 10 mM)  
15 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<sub>2</sub> to the samples b) (PBS)  
20 induced aggregation/flocculation under all conditions, supposedly due to formation of calcium phosphate particles.

These results demonstrate that in buffered solutions such  
25 as i.e. in PBS buffer and/or in the presence of CaCl<sub>2</sub>, 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.  
30 Under physiological conditions (i.e. pH 7.4; 2.2 mM Ca<sup>2+</sup>), 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.  
35

## RNA FORMULATION FOR IMMUNOTHERAPY

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

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

## (56) References Cited

| U.S. PATENT DOCUMENTS |         | References Cited |  |
|-----------------------|---------|------------------|--|
| 4,897,355 A           | 1/1990  | Eppste           |  |
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| 5,703,055 A           | 12/1997 | Felgner          |  |
| 6,251,399 B1          | 6/2001  | Diamant          |  |
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| 6,586,410 B1          | 7/2003  | Wheeler et al.   |  |
| 7,203,981 B2          | 12/2007 | Huang et al.     |  |
|                       |         | Sette et al.     |  |
|                       |         | Gregor et al.    |  |

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

...“Sfortunatamente, per liposomi e nanoparticelle lipidiche aventi **carica positiva** è stata segnalata **un'elevata tossicità**, che può essere un **problema per l'applicazione di tali preparati come prodotti farmaceutici...**”

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.

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.

...“L'uso del tampone pH, spesso necessario per le applicazioni farmaceutiche, e la presenza di composti ionici, sono fattori che possono portare all'aggregazione delle nanoparticelle lipidiche e sono dunque da ritenersi inadatti per applicazioni parenterali...”

...“In presenza del tampone pH denominato PBS, lo stesso effetto è ancor più evidente. Le nanoparticelle lipidiche con un rapporto di carica positiva o neutra formano particelle più grandi.”

RNA FORMULATION FOR  
IMMUNOTHERAPY

BIONTECH RNA PHARMACEUTICALS  
GMBH

## UNA COMPOSIZIONE COLLOIDALE A BASE DI mRNA DESTINATA ALL'IMMUNOTERAPIA:

1. **NON dovrebbe contenere** nella propria formulazione **un tampone ionico come il PBS**, al fine di prevenire fenomeni di **aggregazione, agglomerazione, flocculazione** delle nanoparticelle lipidiche, con tutte le conseguenze di ordine tossicologico che ciò comporta.
2. **NON dovrebbe contenere** nella propria formulazione **composti ionici** (come ad es. cloruro di sodio), al fine di prevenire fenomeni di **aggregazione, agglomerazione, flocculazione** delle nanoparticelle lipidiche, con tutte le conseguenze di ordine tossicologico che ciò comporta.
3. **NON dovrebbe essere iniettato per via parenterale**, a causa della sua **instabilità** quando viene a trovarsi **nelle condizioni fisiologiche del distretto extracellulare** (pH 7,4; 2,2 mM Ca<sup>++</sup>)

## 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

## **IL PREPARATO MEDICINALE COMIRNATY/** **PBS SUCROSE DI PFIZER/BIONTECH:**

- 1. CONTIENE un tampone ionico come il PBS**
- 2. CONTIENE composti ionici (come ad es. cloruro di sodio)**
- 3. VIENE INOCULATO per via parenterale**

**BREVETTO BIONTECH US 10,485,884 B2**

**Concesso 26 NOV 2019**

**Domanda PCT: 25 MAR 2013**

**VACCINO PFIZER/ BIONTECH Comirnaty**

**(Formulazione PBS/Sucrose)**

**AUTORIZZATO DA EMA IL 21 DIC 2020**

[12] **United States Patent**  
Sahin et al. (10) Patent No.: **US 10,485,884 B2**  
(45) Date of Patent: **Nov. 26, 2019**

(54) **RNA FORMULATION FOR IMMUNOTHERAPY** (56) **References Cited**  
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(71) Applicants: **BIONTECH RNA PHARMACEUTICALS GMBH, Mainz (DE); TRON-TRANSLATIONALE ONKOLOGIE AN DER UNIVERSITÄTSMEDIZIN DER JOHANNES GUTENBERG-UNIVERSITÄT MAINZ GEMEINNÜTZIGE GMBH, Mainz (DE)**  
(72) Inventors: **Ugur Sahin, Mainz (DE); Heinrich Haas, Mainz (DE); Sebastian Kreiter, Mainz (DE); Mustafa Dilken, Mainz (DE); Daniel Fritz, Mainz (DE); Martin Meng, Mainz (DE); Lena Mareen Kranz, Mainz (DE); Kerstin Reuter, Darmstadt (DE)**

(\*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.  
(21) Appl. No.: **14/388,192**  
(22) PCT Filed: **Mar. 25, 2013**

| Ingrediente                   | Funzione        | Quantità per |
|-------------------------------|-----------------|--------------|
| mRNA (BNT162b2)               | Attivo          |              |
| ALC-0315                      | Lipide funz     |              |
| ALC-0159                      | Lipide funz     |              |
| DSPC                          | Lipide stru     |              |
| Colesterolo                   | Lipide stru     |              |
| Saccarosio                    | Crioprote       |              |
| Cloruro di sodio              | Elettrolita: co |              |
| Cloruro di potassio           | tampone         |              |
| Fosfato disodico diidrato     | Elettrolita: co |              |
| Diidrogenofosfato di potassio | tampone         |              |



**NO  
ELETTROLITI**

**SI  
ELETTROLITI**

**BREVETTO BIONTECH US 10,485,884 B2**

**Concesso 26 NOV 2019**

**Domanda PCT: 25 MAR 2013**

**VACCINO PFIZER/ BIONTECH Comirnaty**

**(Formulazione PBS/Sucrose)**

**AUTORIZZATO DA EMA IL 21 DIC 2020**

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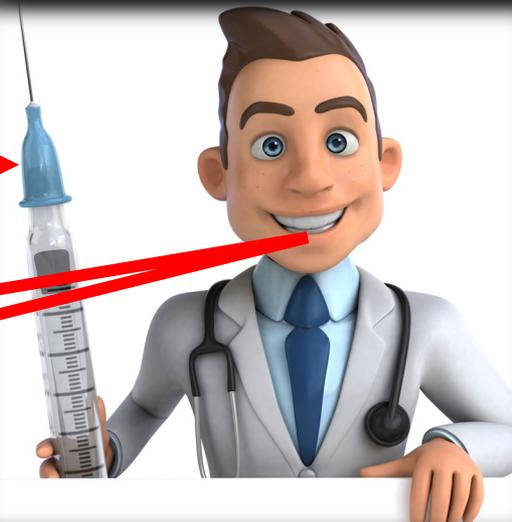
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| 6,586,410 B1      | 7/2003  | Wheeler et al.                     |
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|                   | 12/2011 | Sahin ..... A61K 39/39 424/204.1   |
|                   |         | Schnum et al.                      |
|                   |         | Fougerolles et al.                 |



| Funzione                           | Quantità per dose |
|------------------------------------|-------------------|
| Attivo                             | 30 µg             |
| Lipide funzionale                  | 0,43 mg           |
| Lipide funzionale                  | 0,05 mg           |
| Lipide strutturale                 | 0,09 mg           |
| Lipide strutturale                 | 0,2 mg            |
| Crioprotettivo                     | 6 mg              |
| Elettrolita: componente tampone pH | 2,52 mg           |
| Elettrolita: componente tampone pH | 0,01 mg           |
| Elettrolita: componente tampone pH | 0,07 mg           |
| Elettrolita: componente tampone pH | 0,01 mg           |



**SICURO & EFFICACE**



mRNA: 30  $\mu\text{g}$ / dose



**A**

mRNA: 30  $\mu\text{g}$ / dose



**B**

mRNA: 30  $\mu\text{g}$ / dose



**C**

**mRNA per dose:  
30  $\mu\text{g}$  in 0.3 ml**

**SOSPENSIONE  
OMOGENEA**

**mRNA per  
dose: 30  $\mu\text{g}$**

mRNA: 60  $\mu\text{g}$ / dose



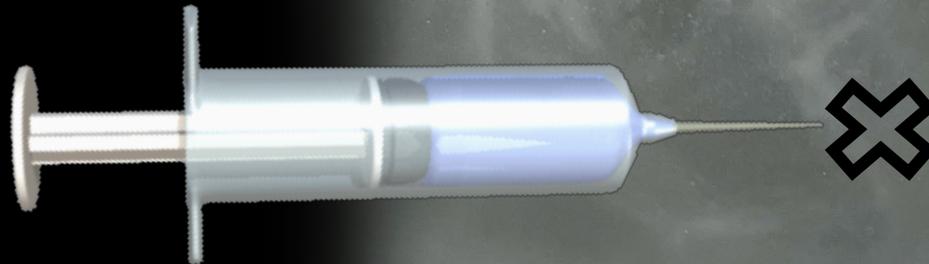
**A**

mRNA: 20  $\mu\text{g}$ / dose



**B**

mRNA: 10  $\mu\text{g}$ / dose



**C**

**Aggregazioni  
Agglomerazioni**

**mRNA per  
dose: 30  $\mu\text{g}$**

**A**



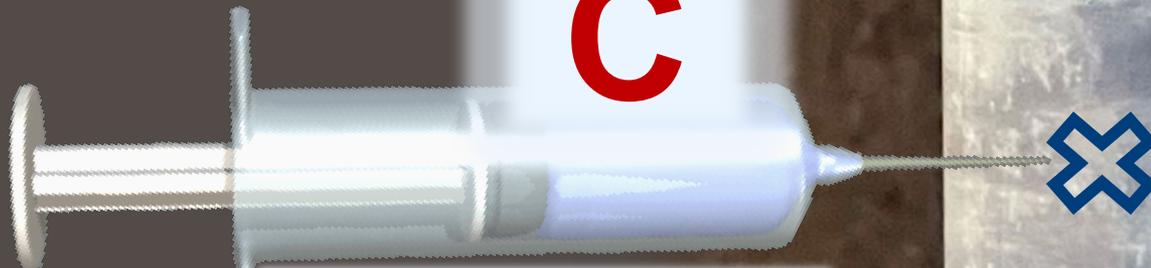
**mRNA: 5  $\mu\text{g}$ / dose**

**B**



**mRNA: 15  $\mu\text{g}$ / dose**

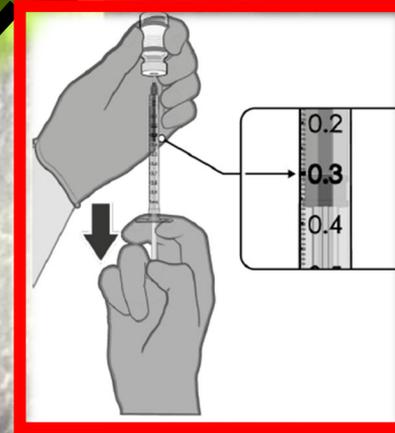
**C**



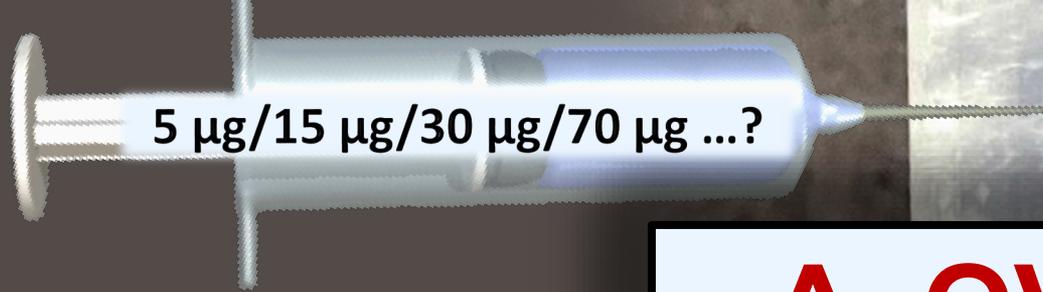
**mRNA: 70  $\mu\text{g}$ / dose**

**Flocculazioni  
Coalescenze**

QUANTITÀ PREVISTA di mRNA per dose: **30  $\mu\text{g}$**



**30  $\mu\text{g}$  = 30 MILIONESIMI DI  
GRAMMO  
= 0,000030 GRAMMI**

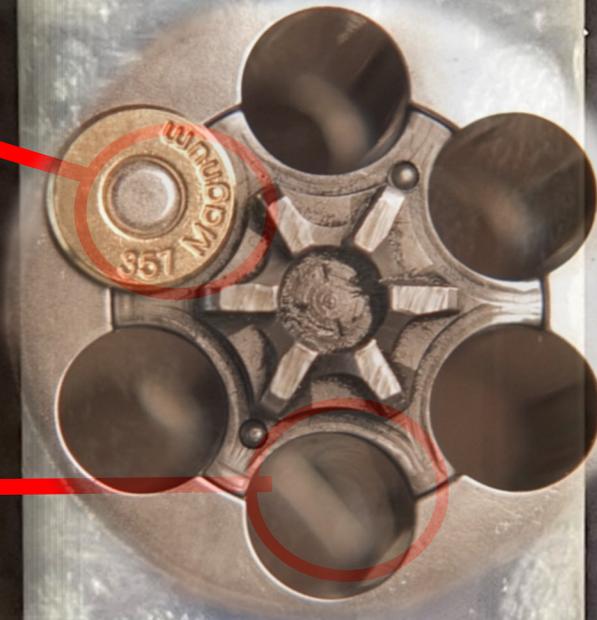


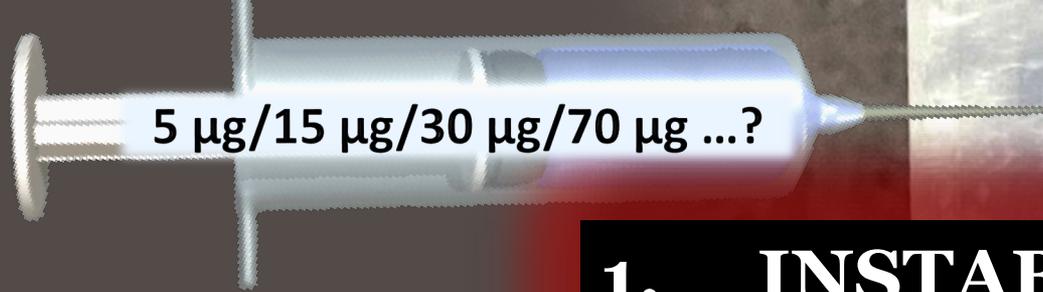
**A. OVERDOSE**  
**B. UNDERDOSE**



**A. MAGGIOR  
CITOTOSSICITÀ E  
IMMUNOREATTIVITÀ**

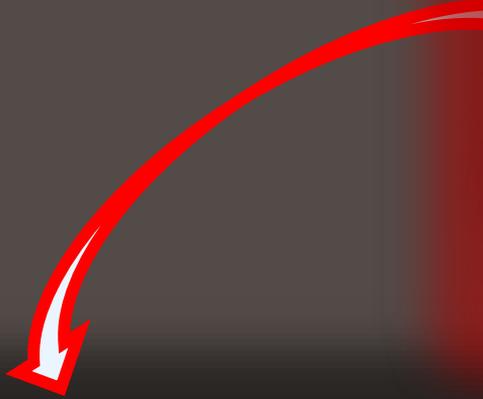
**B. PARZIALE O TOTALE  
INEFFICACIA**





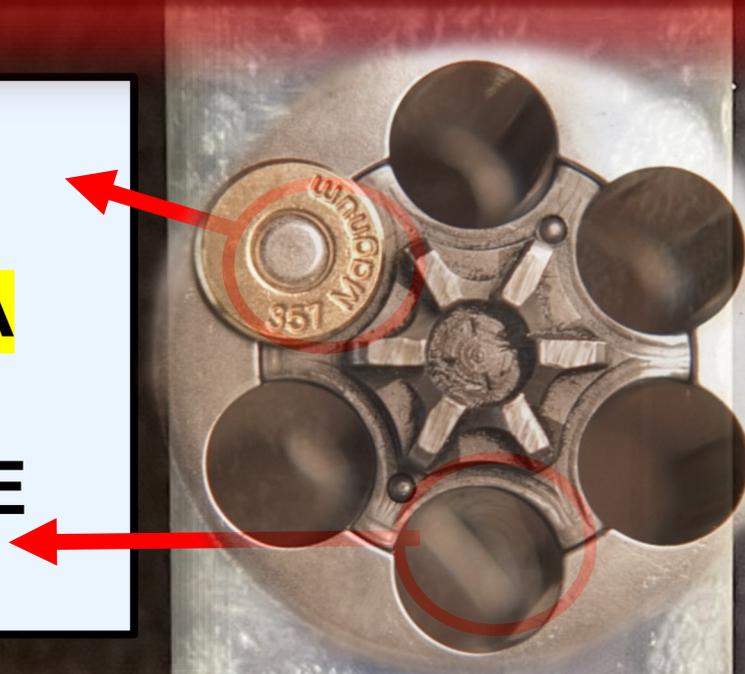
1. **INSTABILE**
2. **INSICURO**
3. **INEFFICACE**

**MEDICINALE IMPERFETTO**  
**(Art. 443 C.P.)**



**A. MAGGIOR  
CITOTOSSICITÀ E  
IMMUNOREATTIVITÀ**

**B. PARZIALE O TOTALE  
INEFFICACIA**



Come «RISOLVERE» I PROBLEMI DI TRASPORTO E  
STOCCAGGIO di un sistema colloidale termodinamicamente  
INSTABILE ?



**INSTABILE !**

# Come «RISOLVERE» I PROBLEMI DI TRASPORTO E STOCCAGGIO di un sistema colloidale termodinamicamente

INSTABILE ?



- 72°C

37°C

25°C

T. ambiente



-72°C

$\Delta T = 109\text{ }^{\circ}\text{C}$

$\Delta T = 97\text{ }^{\circ}\text{C}$

# ULTERIORI RISCHI DI COAGULAZIONE

**COAGULAZIONE** in Chimica:

«La coagulazione può essere provocata dal **riscaldamento** ma soprattutto dall'azione di agenti chimici, in particolare gli **elettroliti**, che alterano la forza ionica della soluzione»

**(ENCICLOPEDIA TRECCANI)**  
[coagulazione nell'Enciclopedia Treccani](#)





EUROPEAN MEDICINES AGENCY  
SCIENCE MEDICINES HEALTH

«...Il CHMP ha approvato **una formulazione pronta all'uso di Comirnaty**. Questa formulazione **non richiede diluizione prima della somministrazione**, sarà disponibile in confezioni da 10 flaconcini (60 dosi) e **può essere conservata a 2-8°C per un massimo di 10 settimane**. **L'attuale formulazione concentrata richiede la diluizione prima della somministrazione**, è disponibile in una confezione da 195 flaconcini (1.170 dosi) e può essere conservata a 2-8°C per un massimo di un mese).

Queste differenze forniranno **migliori opzioni di stoccaggio, trasporto e logistica** per la distribuzione e la somministrazione del vaccino. La nuova formulazione sarà disponibile in un lancio graduale a partire dall'inizio del 2022.»



# 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

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 finished product.

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

**TRIS/Sucrose**

**Capsula di chiusura grigia**



**Table 2 Composition of BNT162b2 Tris/Sucrose Finished Product, Multi-dose 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 <sup>b</sup>     |
| 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.                   |

**ELIMINATI TUTTI GLI ELETTROLITI**



**«MIGLIORE STABILITÀ»**

### ***Formulation development***

The development of the **Tris/Sucrose finished product** showed quality attributes highly comparable and also within the clinical ranges of the current approved PBS/Sucrose finished product but with an **improved stability profile** in support of **increased storage times** at -20 °C and 2-8 °C that would **simplify transport and administration**.

The proposed Tris/Sucrose formulation is equivalent to the current PBS/Sucrose formulation but with the following differences: **formulation buffer system (Tris vs PBS), mRNA concentration (0.1 mg/mL vs 0.5 mg/mL)** and with an advantage that **the proposed Tris/Sucrose finished product does not require dilution upon administration**.

| Formulazione  | Soggetti di età pari o superiore a 12 anni,<br>Da diluire per l'uso   | Soggetti di età pari o superiore a 12 anni,<br>Già diluito per l'uso  | Bambini di età compresa fra 5 e 11 anni,<br>Da diluire per l'uso  |
|---|---|---|---|
| Flaconcino  | Flaconcino multidose  | Flaconcino multidose  | Flaconcino multidose  |
| Colore tappo del flaconcino                                     | Viola<br>                  | Grigio<br>                               | Arancione<br>                            |
|   | <b>PBS/Sucrose</b>  | <b>TRIS/Sucrose</b>   |   |
| Riassunto delle Caratteristiche del Prodotto                    | <u>Caratteristiche del Prodotto</u><br><a href="http://www.COMIRNATYglobal.com">www.COMIRNATYglobal.com</a> | <u>Riassunto delle Caratteristiche del Prodotto</u><br><a href="http://www.COMIRNATYglobal.com">www.COMIRNATYglobal.com</a> | <u>Riassunto delle Caratteristiche del Prodotto</u><br><a href="http://www.COMIRNATYglobal.com">www.COMIRNATYglobal.com</a> |
| Dose  | 30 mcg  | 30 mcg  | 10 mcg  |
| Diluizione  | Diluizione necessaria   | Diluizione <b>NON</b> necessaria  | Diluizione necessaria   |
| Quantità di diluente necessaria per flaconcino                  | 1,8 mL  | Nessuna diluizione  | 1,3 mL  |
| Dimensione del flaconcino                                       | 2 mL  | 2 mL  | 2 mL  |
| Dosi per flaconcino   | 6 dosi per flaconcino (dopo diluizione)   | 6 dosi per flaconcino   | 10 dosi per flaconcino (dopo diluizione)  |
| Volume di iniezione per dose                                    | 0,3 mL  | 0,3 mL  | 0,2 mL  |
| Volume di riempimento per flaconcino                            | 0,45 mL   | 2,25 mL   | 1,3 mL  |
| Tempo di conservazione in frigorifero (tra 2 °C e 8 °C)         | 1 mese  | 10 settimane  | 10 settimane  |
| Tempo di conservazione in congelatore (tra -25 °C e -15 °C)     | Durata massima di 2 settimane durante il periodo di validità di 9 mesi                                      | NON CONSERVARE  | NON CONSERVARE  |
| Tempo di conservazione in congelatore ULT (tra -90 °C e -60 °C) | 9 mesi (periodo di validità)  | 9 mesi (periodo di validità)  | 9 mesi (periodo di validità)  |
| Temperatura ambiente (tra 8 °C e 30 °C)                         | 2 ore prima della diluizione (compreso il tempo di scongelamento)   | 12 ore prima della prima perforazione   | 12 ore prima della diluizione   |
| Dopo prima perforazione o diluizione (tra 2 °C e 30 °C)         | Gettare dopo 6 ore dalla diluizione   | Gettare dopo 12 ore dalla prima perforazione  | Gettare dopo 12 ore dalla diluizione  |
| Dimensione della confezione                                     | 195 flaconcini  | 10 flaconcini   | 10 flaconcini   |

# **PSUR # 3**

## **PERIODIC SAFETY UPDATE REPORT #3**

for

**ACTIVE SUBSTANCE: COVID 19 mRNA vaccine (nucleoside modified) (BNT162b2)<sup>1</sup>**

**ATC CODE: J07BX03<sup>2</sup>**

**AUTHORISATION PROCEDURE in the EU: Centralised**

**INTERNATIONAL BIRTH DATE (IBD)<sup>3</sup>: 19 DECEMBER 2020**

**EUROPEAN UNION REFERENCE DATE (EURD): 19 DECEMBER 2020**

**INTERVAL COVERED BY THIS REPORT:**

**19 DECEMBER 2021 through 18 JUNE 2022**

**DATE OF THIS REPORT: 18 AUGUST 2022**

**Table 20. Demographic Information – Comparison of Paediatric ( $\leq 17$  years) Tris/Sucrose (Grey and Orange Cap) versus Paediatric PBS/Sucrose Cases**

**INTERVAL COVERED BY THIS REPORT:  
19 DECEMBER 2021 through 18 JUNE 2022**

**PSUR # 3**



|  |                           | <b>Tris/Sucrose (Grey and Orange Cap)</b><br>No. of Cases <sup>a</sup> (%) <sup>b</sup><br>N=9055 | <b>PBS/Sucrose</b><br>No. of Cases (%)<br>N=22,772 |
|--|---------------------------|---|--|
| MC cases                               | Yes                       | 6471 (71.5)   | 13,659 (60.0)                                      |
|  | No                        | 2584 (28.5)   | 9113 (40.0)  |
| Country of occurrence                  | Germany                   | 773 (8.5)   | 3916 (17.2)  |
|  | Philippines               | 1229 (13.6)   | 3064 (13.5)  |
|  | Australia                 | 1508 (16.7)   | 2155 (9.5)   |
|  | Malaysia                  | 7 (0.08)  | 1190 (5.2)   |
|  | Taiwan, Province of China | 10 (0.1)  | 1188 (5.2)   |
|  | France                    | 102 (1.1)   | 1080 (4.7)   |
|  | US                        | 2537 (28.0)   | 1009 (4.4)   |
|  | Japan                     | 835 (9.2)   | 510 (2.2)  |
|  | Italy                     | 406 (4.5)   | 634 (2.8)  |
|  | Spain                     | 377 (4.2)   | 370 (1.6)  |
|  | Denmark                   | 146 (1.6)   | 160 (0.7)  |
|  | Canada                    | 134 (1.5)   | 175 (0.8)  |
|  | Ireland                   | 121 (1.3)   | 171 (0.8)  |
|  | Total Others              | 870 (9.6)   | 7150 (31.4)  |
|  | Gender                    | Female  | 3804 (42.0)  |
| Male                                   |                           | 4078 (45.0)   | 10,012 (44.0)                                      |
| Unknown/No Data                        |                           | 1173 (13.0)   | 864 (3.8)  |
| Age (years)                            | N                         | 8213  | 22,089   |
|  | Min-Max                   | 0.58-17   | 0.01-17  |
|  | Mean / Median             | 8.7/9   | 14.3/15  |
| Age Range                              | 0-27 days                 | 0   | 3 (0.01)   |
|  | 28 days to 23 months      | 11 (0.1)  | 69 (0.3)   |
|  | 2-11 years                | 8390 (92.7)   | 1410 (6.2)   |
|  | 12-17 years               | 654 (7.2)   | 21,290 (93.5)                                      |
| <b>Case Seriousness</b>                | Serious                   | 1589 (17.5)   | 6956 (30.5)  |
|  | Non-serious               | 7466 (82.5)   | 15,816 (69.5)                                      |
| Case Outcome                           | Fatal                     | 17 (0.2)  | 65 (0.3)   |
|  | Not resolved              | 1288 (14.2)   | 5013 (22.0)  |
|  | Resolved/Resolving        | 4205 (46.4)   | 11,567 (50.8)                                      |
|  | Resolved with sequelae    | 24 (0.3)  | 142 (0.6)  |
|  | Unknown/No data           | 3521 (38.9)   | 5985 (26.3)  |
| Presence of comorbidities <sup>c</sup> | Yes                       | 289 (3.2)   | 642 (2.8)  |
|  | No                        | 8766 (96.8)   | 22,130 (97.2)                                      |

**1,8 volte maggiore**

**Table 21. Events Reported in  $\geq 2\%$  Cases - Comparison of Paediatric Tris/Sucrose (Grey and Orange Cap) versus PBS/Sucrose Cases**

**INTERVAL COVERED BY THIS REPORT:  
19 DECEMBER 2021 through 18 JUNE 2022**

**PSUR # 3**

|  |  <b>Tris/Sucrose<br/>(Grey and Orange Cap)</b><br>N=9055<br>n (AERP, %) |  <b>PBS/Sucrose</b><br>N=22,772<br>n (AERP, %) |
|--|---|--|
| <b>MedDRA SOC</b>  |   |  |
| <b>MedDRA PT</b>   |   |  |
| <b>Injury, poisoning and procedural complications<sup>40</sup></b> |   |  |
| Poor quality product administered                                  | 1222 (13.5)   | 289 (1.3)  |
| Product administration error                                       | 888 (9.8)   | 162 (0.7)  |
| Product administered to patient of inappropriate age               | 503 (5.6)   | 1364 (6.0)   |
| Overdose   | 478 (5.3)   | 125 (0.6)  |
| Product preparation error  | 433 (4.8)   | 28 (0.1)   |
| Underdose  | 289 (3.2)   | 241 (1.1)  |
| Inappropriate schedule of product administration                   | 243 (2.7)   | 952 (4.2)  |
| Product preparation issue  | 234 (2.6)   | 99 (0.4)   |
| Expired product administered <sup>b</sup>                          | 215 (2.4)   | 72 (0.3)   |
| Vaccination error  | 208 (2.3)   | 258 (1.1)  |
| <b>General disorders and administration site conditions</b>        |   |  |
| Pyrexia  | 1222 (13.5)   | 3507 (15.4)  |
| Vaccination site pain  | 1032 (11.4)   | 2073 (9.1)   |
| Fatigue  | 371 (4.1)   | 2010 (8.8)   |
| Chest pain   | 334 (3.7)   | 1959 (8.6)   |
| Malaise  | 287 (3.2)   | 1000 (4.4)   |
| Drug ineffective   | 218 (2.4)   | 601 (2.6)  |
| Vaccination failure  | 46 (0.5)  | 1061 (4.7)   |
| Chills   | 143 (1.6)   | 978 (4.3)  |
| Chest discomfort   | 63 (0.7)  | 822 (3.6)  |
| Asthenia   | 117 (1.3)   | 582 (2.6)  |
| Pain   | 161 (1.8)   | 528 (2.3)  |
| <b>Nervous system disorders</b>                                    |   |  |
| Headache   | 909 (10.0)  | 3576 (15.7)  |
| Dizziness  | 355 (3.9)   | 2390 (10.5)  |
| Syncope  | 211 (2.3)   | 685 (3.0)  |
| <b>Gastrointestinal disorders</b>                                  |   |  |
| Vomiting   | 683 (7.5)   | 1275 (5.6)   |
| Nausea   | 386 (4.3)   | 1699 (7.5)   |
| Abdominal pain   | 348 (3.8)   | 506 (2.2)  |
| Diarrhoea  | 236 (2.6)   | 448 (2.0)  |
| <b>Skin and subcutaneous tissue disorders</b>                      |   |  |
| Rash   | 501 (5.5)   | 1037 (4.6)   |
| Urticaria  | 254 (2.8)   | 427 (1.9)  |
| Pruritus   | 234 (2.6)   | 484 (2.1)  |
| <b>Musculoskeletal and connective tissue disorders</b>             |   |  |
| Pain in extremity  | 278 (3.1)   | 768 (3.4)  |
| Myalgia  | 172 (1.9)   | 992 (4.4)  |

**9,4 volte maggiore**

COVID-19 mRNA vaccine (nucleoside modified)  
Periodic Safety Update Report (PSUR) 3

Reporting Period  
19 December 2021 through 18 June 2022

**Table 21. Events Reported in  $\geq 2\%$ <sup>a</sup> Cases - Comparison of Paediatric Tris/Sucrose (Grey and Orange Cap) versus PBS/Sucrose Cases**

|  |  <b>Tris/Sucrose<br/>(Grey and Orange Cap)</b> |  <b>PBS/Sucrose</b> |
|--|---|--|
| MedDRA SOC   | N=9055  | N=22,772   |
| MedDRA PT  | n (AERP, %)   | n (AERP, %)  |
| Arthralgia   | 123 (1.4)   | 491 (2.2)  |
| <b>Product issues</b>                                  |   |  |
| Product temperature excursion issue                    | 253 (2.8)   | 114 (0.5)  |
| <b>Respiratory, thoracic and mediastinal disorders</b> |   |  |
| Dyspnoea   | 227 (2.5)   | 1269 (5.6)   |
| Cough  | 172 (1.9)   | 445 (2.0)  |
| <b>Blood and lymphatic system disorders</b>            |   |  |
| Lymphadenopathy  | 203 (2.2)   | 903 (4.0)  |
| <b>Cardiac disorders</b>                               |   |  |
| Palpitations   | 85 (0.9)  | 691 (3.0)  |
| Myocarditis  | 38 (0.4)  | 632 (2.8)  |
| Tachycardia  | 82 (0.9)  | 467 (2.1)  |
| <b>Total number of events</b>                          | <b>14,457</b>   | <b>38,010</b>  |

1,8 volte maggiore

3,4 volte maggiore

7 volte maggiore

2,4 volte maggiore

- a. Reporting proportion (% of total PM cases) in one or both paediatric populations.
- b. Majority of the cases reported uncertain expiry dates.

**Table 20. Demographic Information – Comparison of Paediatric ( $\leq 17$  years) Tris/Sucrose (Grey and Orange Cap) versus Paediatric PBS/Sucrose Cases**

**INTERVAL COVERED BY THIS REPORT:  
19 DECEMBER 2021 through 18 JUNE 2022**

**PSUR # 3**

|  |                           |  <b>Tris/Sucrose (Grey and Orange Cap)</b><br>No. of Cases <sup>a</sup> (%) <sup>b</sup><br>N=9055 |  <b>PBS/Sucrose</b><br>No. of Cases (%)<br>N=22,772 |               |
|--|---------------------------|---|--|---------------|
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|  | Italy                     | 406 (4.5)   | 634 (2.8)  |               |
|  | Spain                     | 377 (4.2)   | 370 (1.6)  |               |
|  | Denmark                   | 146 (1.6)   | 160 (0.7)  |               |
|  | Canada                    | 134 (1.5)   | 175 (0.8)  |               |
|  | Ireland                   | 121 (1.3)   | 171 (0.8)  |               |
|  | Total Others              | 870 (9.6)   | 7150 (31.4)  |               |
|  | Gender                    | Female  | 3804 (42.0)  | 11,896 (52.2) |
|  |                           | Male  | 4078 (45.0)  | 10,012 (44.0) |
| Unknown/No Data                        |                           | 1173 (13.0)   | 864 (3.8)  |               |
| Age (years)                            | N                         | 8213  | 22,089   |               |
|  | Min-Max                   | 0.58-17   | 0.01-17  |               |
|  | Mean / Median             | 8.7/9   | 14.3/15  |               |
|  | <b>Age Range</b>          |   |  |               |
| Case Seriousness                       | 0-27 days                 | 0   | 3 (0.01)   |               |
|  | 28 days to 23 months      | 11 (0.1)  | 69 (0.3)   |               |
|  | 2-11 years                | 8390 (92.7)   | 1410 (6.2)   |               |
|  | 12-17 years               | 654 (7.2)   | 21,290 (93.5)  |               |
| Case Outcome                           | Serious                   | 1589 (17.5)   | 6956 (30.5)  |               |
|  | Non-serious               | 7466 (82.5)   | 15,816 (69.5)  |               |
| Presence of comorbidities <sup>c</sup> | Fatal                     | 17 (0.2)  | 65 (0.3)   |               |
|  | Not resolved              | 1288 (14.2)   | 5013 (22.0)  |               |
|  | Resolved/Resolving        | 4205 (46.4)   | 11,567 (50.8)  |               |
|  | Resolved with sequelae    | 24 (0.3)  | 142 (0.6)  |               |
|  | Unknown/No data           | 3521 (38.9)   | 5985 (26.3)  |               |
| Case Outcome                           | Yes                       | 289 (3.2)   | 642 (2.8)  |               |
|  | No                        | 8766 (96.8)   | 22,130 (97.2)  |               |

**3 (0,01%)  
SOGGETTI  
Età < 27 giorni**

**69 (0,3%)  
SOGGETTI  
Età < 2 anni**

**1410 (6,2%)  
SOGGETTI  
Età 2-11 anni**

**Conclusion**

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Overall, more than 45% of the Tris/Sucrose cases was reported in paediatric subjects; the most frequently reported AEs in this population do not differ from the paediatric PBS/Sucrose formulation. A higher percentage of medication error cases was reported in the Tris/Sucrose paediatric group and this may reflect initial difficulties in managing the new formulation (Section 9.2, *Medication Errors*). Routine pharmacovigilance activities to mitigate these medication errors are listed in the approved version 5.0 of the EU-RMP adopted on 10 March 2022.

Based on the review of the cases reported with Tris/Sucrose formulation, no new safety issues were identified.

**CONCLUSIONE**

***...Sulla base della revisione dei casi riportati con la formulazione Tris/Sucrose, non sono stati identificati nuovi problemi di sicurezza.***

## Conclusion

Overall, more  
most frequen  
PBS/Sucrose  
Tris/Sucrose  
formulation (C  
mitigate these  
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Based on the  
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safety

## CONCLUSIONE

*...Sulla base della revisione dei casi riportati  
**con la formulazione Tris/Sucrose, non sono**  
**stati identificati nuovi problemi di sicurezza.***





# **CODICE DI NORIMBERGA**

## **ARTICOLO 5**

**Non si deve eseguire la  
sperimentazione se a priori si è a  
conoscenza che tale sperimentazione  
possa causare danni o morte...**



# **CODICE DI NORIMBERGA**

## **ARTICOLO 10**

**Durante l'esperimento, lo scienziato responsabile deve essere pronto a interromperlo in qualunque momento se indotto a credere che la continuazione dell'esperimento comporterebbe probabilmente lesioni, invalidità o morte per il soggetto umano.**