Anti-PEG syndrome: the impact of biotechnology on immunothrombosis

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SUMMARY

Anti-PEG syndrome is an immunothrombotic adverse event characterized by complement activation and mediated by anti-PEG antibodies, which are typically generated through prior exposure to pharmaceutical formulations containing polyethoxylated compounds such as polyethylene glycol (PEG), polysorbates, and others.

Its current relevance lies in the high prevalence of anti-PEG antibodies in the population, driven by the rise of biotechnology, and in the fact that these antibodies exhibit a dual nature of reactivity:

- **Synthetic Reactivity**: Anti-PEG antibodies can interact with the polyethoxylated structures of the widely used chemical compounds that induce their production, leading to various thrombotic adverse effects.

- **Biological Reactivity**: Anti-PEG antibodies can cross-react with mannosylated glycoproteins on the surface of various microorganisms, leading to severe COVID-19 and other forms of infectious immunothrombosis.

Anti-PEG syndrome can manifest across a wide range of time after the initial antigen exposure, which may complicate the identification of its immunological etiology.

Key words: polyethylene glycol (PEG), polysorbate, anti-PEG antibodies, anti-PEG syndrome, mannosylated glycoproteins, complement, immunothrombosis, COVID-19.

1. INTRODUCTION: CURRENT RELEVANCE OF THROMBOSIS AND IMMUNOTHROMBOSIS

In recent years, the incidence of several thrombotic conditions has increased, which has traditionally been attributed to imbalances in the coagulation and fibrinolysis systems.

In contrast to this paradigm, recent research shows that the immune system plays a crucial role in thrombus formation through immunothrombosis, a process that links innate immunity, platelet activation, and coagulation. It serves as an important defense mechanism to prevent pathogens from spreading in the bloodstream, but abnormal activation of this process can raise the risk of cardiovascular events, as observed in COVID-19 (1).

During the pandemic, it was observed that the immune system not only responded to the infectious agent but, in many cases, triggered a coagulation cascade that resulted in

thrombus formation in multiple organs, significantly complicating the clinical condition of patients. Complement and coagulation activation correlated with disease severity (2).

Immunothrombosis is beneficial when there is a local barrier disruption, as in skin wounds. However, in systemic infections, dysregulated immunothrombosis causes systemic coagulopathy and multi-organ failure due to the lack of blood supply to tissues. The interaction between platelets and immune cells is facilitated by the coagulation and complement systems, creating an interrelated process that connects inflammation and thrombosis (1).

While immunothrombosis is activated in the context of bacterial or viral infection, it can also be triggered by the immune system in response to medications, as seen in PEG-induced pseudoallergy (3), heparin-induced thrombocytopenia (4), or immune thrombotic thrombocytopenia induced by the COVID-19 vaccine (5).

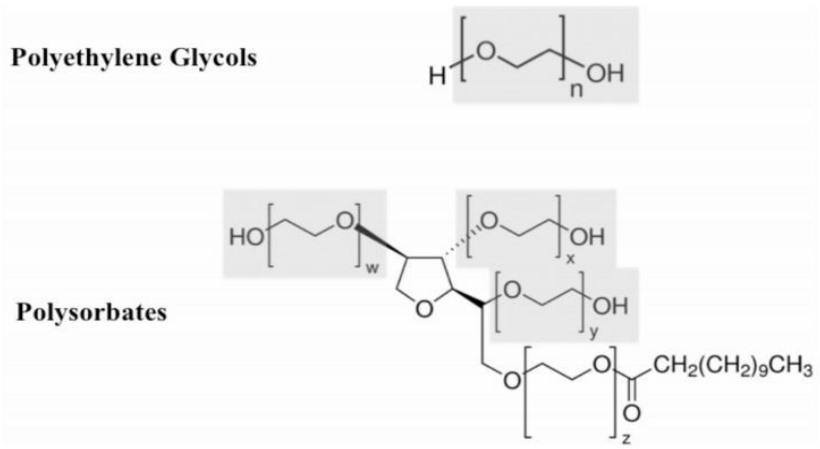
The immune system's reaction to man-made substances like drugs or excipients highlights the interaction between the biological and synthetic worlds and the risks associated with biotechnological advances.

2. ROLE OF PEG AND POLYSORBATES IN CURRENT BIOTECHNOLOGY

In today's fields of biotechnology and pharmaceutical technology, PEGs and polysorbates play a fundamental role in developing new drug formulations, where they are widely used as excipients to improve solubility, stability, and bioavailability of active ingredients.

PEGs are synthetic polymers composed of a repetitive chain of ethylene oxide units. They are used in topical pharmaceutical formulas, laxatives, drug pegylation to prolong their half-life in the body, and more recently in the formulation of lipid nanoparticles within RNA technology.

Polysorbates are synthetic surfactants widely used as stabilizers and emulsifiers in the pharmaceutical industry in vaccines, injectable drugs, and gene therapy formulations. Chemically, polysorbates consist of a sorbitan molecule linked to four short PEG chains, one of which is attached to a fatty acid (6). In other words, PEGs are part of polysorbates.



Both polysorbate 20 (Tween 20) and polysorbate 80 (Tween 80), the most commonly used ones, contain short PEG chains with a hydroxylated end in their structures (7). Additionally, there is structural homology between PEG and polysorbates, and other non-ionic polyoxyethylene-type detergents (8). Among them are Octoxinol-9 (Triton X-100), Poloxamer 188, and polyoxyl castor oil (Cremophor).

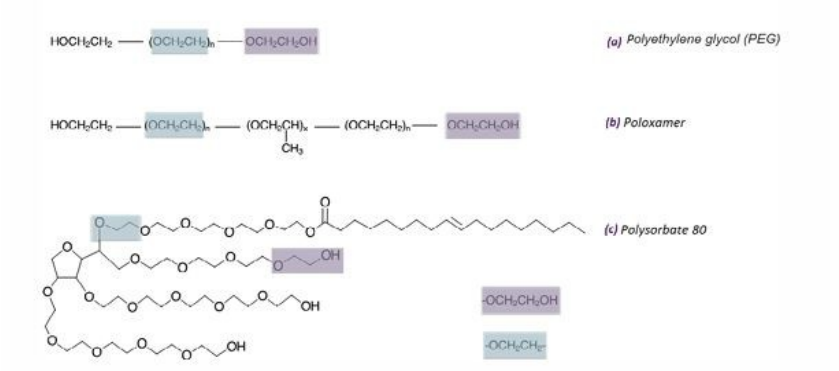
Before the widespread use of mRNA COVID-19 vaccines, PEG was practically only injected with pegylated drugs. Conversely, polysorbates have been widely injected in vaccines such as flu vaccines, human papillomavirus vaccines, and many other biological products (6).

Cross-reactivity between PEG and polysorbates has been observed due to their shared chemical structure, so patients allergic to polysorbates should be carefully monitored when receiving mRNA COVID-19 vaccines containing PEG (9).

3. ORIGIN AND PRODUCTION OF ANTI-PEG ANTIBODIES

From an immunological standpoint, PEG and polysorbates share polyether groups as a common repetitive chemical epitope, resulting in cross-reactions and the generation of the same anti-PEG antibodies (10).

Anti-PEG antibodies are produced in response to the repetitive synthetic structures of ethylene oxide found on the surface of micelles formed by polysorbates, octylphenols, and PEGylated nanoparticles. As illustrated by Wenande, the chemical groups $–(OCH_2CH_2)–$ and OCH_2CH_2OH are shared by PEG and its derivatives, such as polysorbates and poloxamers, enabling cross-sensitization (11).



IgM anti-PEG antibodies are generated in the spleen through a T-cell independent type 2 (TI-2) process. During this process, PEGs come into contact with marginal zone B cells, cross-linking their surface antibodies (12).

Anti-PEG antibodies induced by the HO-PEG-protein complex are directed against the main structure of the polymer (7). In fact, the AGP3 antibody, which targets the main structure of PEG, has been shown to inhibit various pegylated therapies to different extents (13).

The already extensive literature on anti-PEG antibodies and their role in immune-mediated side effects from pegylated drugs (14) has expanded significantly with the widespread use of COVID-19 vaccines (15).

4. CURRENT EXTENT AND PREVALENCE OF ANTI-PEG ANTIBODIES

Recent research has revealed the high prevalence of anti-PEG antibodies in the general population, highlighting the significant extent to which biotechnology affects our immune system. A pre-pandemic study revealed that up to 72% of healthy individuals had anti-PEG antibodies, and 7% had levels high enough to predispose them to anaphylactic reactions (16).

Later data show that the use of PEG in mRNA lipid nanoparticle vaccines against COVID-19 is associated with the induction of anti-PEG antibodies in healthy individuals, further contributing to the development or reinforcement of pre-existing antibodies and increasing the risk of antibody-mediated toxicities for other PEG-containing products (17).

Another post-pandemic study confirmed the presence of IgG anti-PEG antibodies across the entire cohort, with significant variability between individuals, and 12.5% of them had levels above 1000 AU/mL, which could be considered a concerning threshold for anaphylactic reactions (18).

Pre-existing anti-PEG antibodies have been detected in healthy people who have never been treated with pegylated therapies (19). Since the anti-PEG ELISA test does not distinguish between antibodies generated by PEG or polysorbate due to cross-reactivity (20), the high rate of anti-PEG antibodies observed in the general population can be explained by the high exposure to polysorbates present in a variety of widely used injectable products such as flu and human papillomavirus vaccines, monoclonal antibodies, insulin, depot corticosteroids, amiodarone, etc. (Table 1).

Table 1

Polyethoxylated compound	Drugs
Polysorbates	Monoclonal antibodies (Avastim, Erbitux, Humira, Imukin, Lemtrada, Mabthera, Remicade...);
	Vaccines (Arexvy, Chiromas, Fluarix, Gardasil, Imovax, Prevenar, Shingrix); Insulins (Lantus); Benefix,
	Eprex, Etoposide, NeoRecormon, Neupogen, Refacto, Remicade, Taxotere, Torisel, Trangorex, Trigon...
Polysorbates and PEG	Adynovi, Depo-Progevera, Jivi, Pegasys, Pegintron, Plegridy, Trevicta, Xeplion
PEG	Caelyx, Cimzia, Comirnaty, Neulasta, Oncaspar, Somavert, Sonovue, Spikevax
Cremophor	Sandimmun, Taxol
Poloxamer	Hormones (Norditropin, Omnitrope, Ovitrelle, Sandostatin); Monoclonal antibodies (Gazyvaro)
Octoxinol	Vaccines (EflUelda, Mutagrip, Vaxigrip)

The high prevalence of anti-PEG antibodies may have gone unnoticed because they can be present without evident clinical manifestations and often escape detection in routine diagnostic tests. Additionally, being polyclonal antibodies, ELISA results show variations depending on the assay conditions of each laboratory (20).

5. COMPLEMENT ACTIVATION BY ANTI-PEG ANTIBODIES: THE ANTI-PEG SYNDROME

Regardless of the antigen that induced their production, anti-PEG antibodies can interact with various compounds sharing polyethoxylated chains. Thus, in the presence of anti-PEG antibodies, a new administration of PEG can trigger a well-documented anaphylactoid reaction mediated by complement activation (3).

Given the high prevalence of these antibodies and the widespread use of injectable substances containing polyethoxylated compounds, their interaction may occur in various situations and generate diverse reactions. In fact, the adverse effects recorded in the technical sheets of several medications containing polysorbates show a pattern of toxicity that points to a common underlying mechanism (Table 2). This allows us to define a syndrome that encompasses them: the anti-PEG syndrome.

The anti-PEG syndrome, therefore, falls under the category of adverse drug reactions (ADR) as a delayed adverse effect, as it appears some time after the first contact with antigens, following subsequent contact with other polyethoxylated compounds. This complicates its identification and makes it possible for it to go unnoticed, with its consequences being mistakenly attributed to other causes.

The immunopathological mechanism of the anti-PEG syndrome revolves around complement system activation, triggered by the formation of immune complexes between anti-PEG antibodies and polyethoxylated chemical chains. As discussed later, these chains can be found in both synthetic and biological compounds, so it's important to distinguish between synthetic and biological anti-PEG syndrome.

Complement activation can occur through the three known pathways: classical, alternative, and lectin (21). As a result, anaphylatoxins are immediately released (3, 22), triggering an inflammatory cascade that can can lead to tissue damage, platelet activation, and thrombus formation. This may result in a hypersensitivity reaction similar to Complement Activation-Related Pseudoallergy (CARPA), as described by Szebeni (3).

To recap, the anti-PEG syndrome encompasses a series of adverse immunological reactions, often delayed in nature, associated with the use of pharmaceutical formulations containing polyethoxylated compounds. These reactions are mediated by anti-PEG antibodies and involve complement activation, leading to immunothrombosis.

6. SYNTHETIC ANTI-PEG SYNDROME (DRUG-INDUCED)

In synthetic anti-PEG syndrome, polyethoxylated compounds present in medications can act both as sensitizing antigens that generate the first antibodies and as effector agents that activate the complement by binding to pre-existing anti-PEG antibodies.

6.1. Synthetic anti-PEG Syndrome due to polysorbates

Flu vaccination is one of the most common ways people can be exposed to polysorbates. In a study where patients received the inactivated flu vaccine with MF59C.1 adjuvant containing polysorbate 80, it was concluded that the increased platelet activation observed after vaccination could temporarily raise the risk of thrombosis in high-risk patients (23).

On the AEMPS (Spanish Agency of Medicines and Medical Devices) website, the technical sheets of several drugs formulated with polysorbates as excipients include various adverse effects of a thrombotic nature attributable to the anti-PEG syndrome (Table 2):

Table 2

Drugs with polysorbate	Thrombotic adverse effect					
	Thrombophlebitis	Deep vein thrombosis	Pulmonary embolism	Myocardial infarction	Stroke	Thrombotic thrombocytopenic purpura (TTP)
Avastim	X	X	X	X	X	
Avonex						X
Benefix	X	X				
Depo-Progevera	X	X	X			
Eprex		X	X	X	X	
Erbitux		X	X	X		
Etoposide				X		
Humira	X		X	X	X	X
Imukin		X	X	X		
Lemtrada				X	X	X
Mabthera				X	X	
NeoRecormon		X		X	X	
Pegasys			X	X	X	
Plegridy						X
Refacto	X					
Remicade	X			X	X	X
Risperdal Consta		X	X		X	
Roferon				X	X	
Simponi		X				
Taxotere				X		
Torisel	X	X				
Trangorex	X					
Trevicta		X				
Trigon	X					
Xeplion		X	X			

Another adverse effect with a possible thrombotic mechanism, such as intestinal perforation, also appears in the technical sheets of drugs containing polysorbates: Adcetris, Avastim, Humira, Kevzara, Remicade, Roactemra, Torisel.

For other formulations containing polysorbate, such as Cosentyx, Ilumetri, Stelara, Taltz, or Tremfya, among others, various thrombotic adverse effects have been reported in VigiAccess, which are not included in their technical sheets.

6.2. Synthetic anti-PEG Syndrome due to PEG

In the case of pegylated therapeutic agents, studies have shown that circulating anti-PEG antibodies selectively bind to PEG molecules, triggering complement system activation. This leads to both the accelerated blood clearance (ABC) phenomenon of pegylated drugs and the appearance of severe adverse effects through pseudoallergic reactions (CARPA) (19).

The technical sheets of several pegylated drugs or those containing PEG as an excipient list adverse effects of a thrombotic nature attributable to the anti-PEG syndrome:

- Caelyx: thrombophlebitis, venous thrombosis, pulmonary embolism.
- Cimzia: thrombophlebitis, pulmonary embolism, stroke.
- Oncaspar: thrombophlebitis, deep vein thrombosis, pulmonary embolism, stroke.
- SonoVue: myocardial infarction.

The thrombotic adverse effects of Pegasys, Pegintron, Plegridy, Trevicta, and Xeplion could be attributed to both PEG and polysorbate, as they contain both.

Regarding vaccines based on the mRNA platform, which include reactions such as pulmonary embolism, thrombosis, cerebral hemorrhage, myocardial infarction, and cerebral venous sinus thrombosis, the pegylated lipid nanoparticles can induce pathogenic anaphylactoid reactions through complement activation and platelet aggregation enhancement, contributing to a higher risk of thromboembolic events (24). The proposed mechanism of complement activation correlates with anti-PEG IgG levels (25).

The reactogenicity of anti-PEG antibodies is well-documented. A study found that individuals with elevated levels of anti-PEG antibodies are at a higher risk of hypersensitivity/anaphylaxis reactions to pegylated vaccines and other pegylated injectable products. This risk could be further increased due to the anti-PEG immunogenicity of these vaccines (20).

6.3. Synthetic anti-PEG Syndrome due to other polyethoxylated drugs

Thrombotic adverse effects attributable to anti-PEG syndrome have been documented in the technical sheets of several drugs with polyethoxylated excipients:

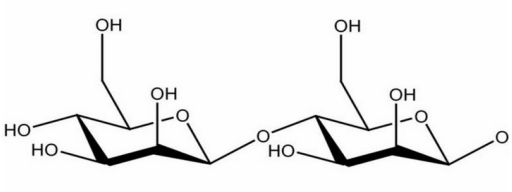
- Microangiopathic thrombosis and thrombotic thrombocytopenic purpura with injectable Sandimmun, which contains polyoxyl castor oil (Cremophor). Also, anaphylactoid reactions with dyspnea after intravenous administration.
- Thrombophlebitis, deep vein thrombosis, pulmonary embolism, and myocardial infarction with Taxol, which contains Cremophor EL.
- Myocardial infarction with Gazyvaro, which contains Poloxamer 188.

7. BIOLOGICAL ANTI-PEG SYNDROME (DUE TO MICROBIAL GLYCOPROTEINS)

Cross-reactions are common among biological antigens such as proteins. However, since the laws of chemistry apply to both synthetic and biological substances, there is a theoretical possibility of cross-reaction between a non-biological antigen and a biological one if they share certain chemical structures with similar or identical epitopes. This is the basis of the biological anti-PEG syndrome, where the effector agents that activate the complement by binding to pre-existing anti-PEG antibodies are polyethoxylated structures present on the surface of many microorganisms.

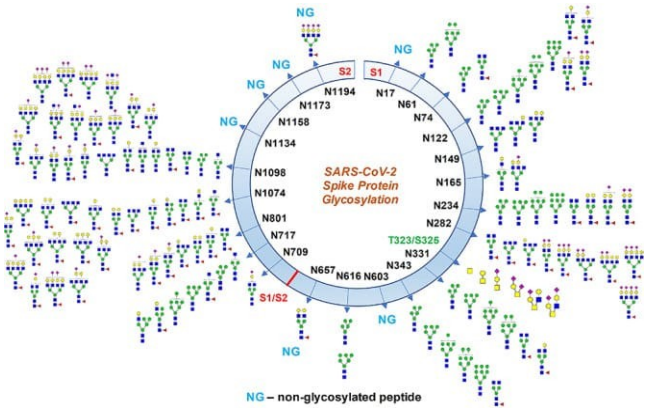
7.1. Biological anti-PEG Syndrome due to mannosylated glycoproteins in severe COVID-19

The repetitive chains of ethylene oxide present on the surface of polysorbate micelles, octylphenols, and pegylated nanoparticles are also found in natural polysaccharides like mannose polymers (mannans) present on the glycoproteins of the external surfaces of various pathogens (26):



Oligomannose

In the case of SARS-CoV-2, the spike protein contains carbohydrate chains in its structure that contain multiple mannose groups (27), represented in the image by green circles:



This abundant presence of mannose groups in the spike protein may allow its immunological recognition in the context of an infection (28), making it susceptible to interaction with anti-PEG antibodies previously generated against similar structures such as PEG chains and polysorbates.

The sequence of the cross-immune reaction can be described as follows:

1. **Initial exposure to synthetic compounds**: The immune system comes into contact with synthetic polyethoxylated compounds present in injected drugs.
2. **Recognition as foreign**: These chemical structures, similar to those of certain microorganisms, are identified as foreign by the immune system.
3. **Generation of anti-PEG antibodies**: In response, the immune system produces antibodies directed against these synthetic polyethoxylated structures.
4. **Subsequent contact with real microorganisms**: During an infection, the body encounters microorganisms that present similar chemical structures to the synthetic compounds.
5. **Cross-reaction**: Anti-PEG antibodies, previously generated against polyethoxylated compounds, recognize the similar structures on the microorganisms, triggering an immune response against them.
6. **Possible pathological immune activation**: This cross-reaction can may cause inflammation, tissue damage, and thrombotic events.

Against common antigenic components, the immune system responds similarly: both the immunological effects caused by polysorbates and the processes observed in severe COVID-19 patients share complement activation as a central mechanism:

- Complement activation plays a crucial role in the genesis of severe COVID-19 through immunothrombosis. In fact, the activation of the complement cascade has been associated with parameters of endothelial damage and necrosis markers, highlighting its essential role in thrombus formation, especially in the pulmonary microcirculation (29). An exaggerated complement deposition is detected in various tissues, including the lungs (30).

- Antibodies can form immune complexes with viruses, which, when deposited, activate the complement system in different tissues, such as the respiratory tract, and trigger cytokine cascades (31).

- Activation is mediated by the classical antibody-dependent pathway in response to elevated levels of circulating immune complexes of IgG and their binding to the C1q protein. This suggests that early IgG antibody responses, which are non-neutralizing, may play a key role in the overactivation of complement in severe COVID-19 cases (32).

These early responses observed in severe COVID-19 cases are consistent with the hypothesis of the pre-existence of anti-PEG antibodies in the patient. The resulting immune process would be similar to CARPA-type reactions triggered by synthetic PEGs, but in this case, the trigger would be viral glycoproteins.

Similarly, the interstitial lung disease observed in severe COVID-19 cases has also been reported as an adverse effect in the technical sheets of several drugs containing polysorbate and/or PEG, such as Cimzia, Erbitux, Etoposide, Humira, Imukin, Neulasta, Neupogen,

Pegasys, Simponi, Stelara, and Taxotere. Both effects may be attributed to anti-PEG syndrome.

The anti-PEG syndrome could also explain why, in older people, the 2019 flu vaccination, which contained polysorbate and generated anti-PEG antibodies, may have become a later risk factor for developing severe COVID-19 (33, 34).

7.2. Biological anti-PEG syndrome due to other viral pathologies

Apart from COVID-19, complement activation has been implicated in various viral diseases, including infections by other coronaviruses and the influenza virus. In these infections, overactivation of the complement system can trigger a maladaptive immune response, leading to a cytokine storm, worsening of the disease, and ultimately cellular and organ dysfunction that can lead to multiple organ failure and even death (35).

Since many of these viruses present mannose polymers on their surface, they can also form complexes with anti-PEG antibodies, which through complement activation could generate a clinical picture similar to severe COVID-19.

On the other hand, there are no distinctive morphological features that can definitively differentiate the diffuse alveolar damage caused by COVID-19 from that produced by other infections. The histopathological findings in patients infected with SARS-CoV-2 show significant overlap, suggesting the existence of common pathogenic mechanisms (36).

Furthermore, the definition of COVID-19 as the syndrome exclusively caused by SARS-CoV-2 was established in the early stages of the pandemic when knowledge about its immunopathology was still limited, which may have led to the over-diagnosis of SARS-CoV-2 as the causal agent in processes generated by other microorganisms.

8. CONCLUSIONS

In recent decades, advancements in biotechnology have led to the extensive use of injectable pharmaceutical formulations containing polyethoxylated compounds such as polysorbates, PEG, octylphenols, Poloxamer, and Cremophor, which induce the production of anti-PEG antibodies. This phenomenon has led to a significant prevalence of anti-PEG antibodies in the global population.

Anti-PEG antibodies, due to their ability to bind to various ligands and activate the complement system, carry a significant risk of triggering immunothrombotic adverse events and severe complications, such as those seen in COVID-19, all of which can be grouped under an emerging clinical entity: the anti-PEG syndrome.

Since the anti-PEG syndrome manifests in a delayed manner, it often goes unnoticed, raising the possibility that it may be involved in thrombotic adverse effects, such as strokes and heart attacks, which are frequently misattributed to atherosclerotic causes.

Likewise, this syndrome could be related to adverse immunological reactions that present respiratory symptoms erroneously associated solely with infectious causes, as seen in cases of "complicated flu".

Recognizing the anti-PEG syndrome has important clinical implications in the context of the growing use of RNA-based therapies, raising the need to investigate how many people develop antibodies against PEG, how long these antibodies persist in circulation, and what their levels are following the administration of such therapies.

For the pharmaceutical and biotechnology industries, the anti-PEG syndrome presents the challenge of developing new formulation strategies that minimize the risk of adverse immunological reactions and ensure greater safety in their products.

Finally, the anti-PEG syndrome could usher in a new approach to understanding immunothrombosis as a severe immune response to exposure to exogenous chemical products like polyethoxylated compounds. Its potential impact on human health highlights the importance of strict and continuous surveillance in assessing the risks associated with their use in pharmaceutical and biotechnological formulations.

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