Are Nitrosamines a Concern for Biologic Manufacturers?

The discovery of nitrosamine genotoxins in several pharmaceuticals has led to monitoring and re-evaluation of manufacturing practices for those products considered at-risk. This article provides an assessment of nitrosamine contamination specifically in biologics and contrasts the potential risks between these product types.

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Foreword

First described over a century ago, the class of organics known as N-nitrosamines are defined by the common structural feature, R^2NNO.

Since the mid-1950s when their carcinogenic nature was revealed (1), this functional class has continued to attract attention by researchers and, today, of the more than 300 nitrosamines identified, 90% are thought to be carcinogenic and/or genotoxic (2).

The presence of nitrosamines in a range of foodstuffs and drinks as well as products such as tobacco is well documented. They are known to form during material preparation as well as in the digestive tract itself (3).

The greatest risk for the presence or formation of N-nitrosamines comes from the confluence of three factors:
(i) A nitrosating agent
(ii) A secondary or tertiary amine, and
(iii) Appropriate conditions (e.g. elevated temperatures, acidic conditions, liquid phase) (4)

Presence of nitrosamines in medicines

In mid-2018, regulators became aware of the presence of the nitrosamine N-nitrosodimethylamine (NDMA) in certain blood pressure medications (5). Further investigations led to the detection of several N-nitrosamine species in a range of pharmaceuticals including valsartan, antibiotics, antacids and antidiabetics. This resulted in the recall of several drug products and the temporary withdrawal of treatment for many patients globally.

In 2019, SGS adopted a center of excellence strategy for nitrosamine testing, utilizing expertise, sophisticated analytical instruments and method harmonization across its global network to support the pharmaceutical industry.

Beginning in late 2020, several additional products were removed from the market due to the detection of nitrosamine drug substance-related impurities (NDSRIs) (6). These are essentially nitroso forms of the API which result from reaction between a nitrosating agent (e.g. nitrite) and a suitable amine/amide-containing API under appropriate chemical conditions (7).

It is estimated that about 20% of current small-molecule generic drugs have a reactive amine functional group which poses the potential for the formation of NDSRIs. As might be expected however, the genotoxicity of such derivatives can vary significantly depending on the molecular structure of the API (8).

Method development and validation by SGS.

SGS has already successfully applied its network of specialized laboratories to tackle the analytical challenges associated with a range of these somewhat unique impurities.

A coordinated regulatory strategy to address potential nitrosamine contamination in existing and new drug products has been established and includes an initial risk assessment followed by appropriate testing and mitigation of the manufacturing processes (9). While much attention was initially focused on traditional pharmaceutical products, in 2020 the EMA published an assessment report (10) that directed all medicinal products, including biologics, into the scope of nitrosamine risk assessment.

Other regulatory agencies such as Health Canada, Swiss Medic and ANVISA have now also emulated this guidance.

The overall portfolio of services provided by SGS is well equipped to support all aspects of these regulatory strategies, from risk assessment and analytical development to evaluation and routine monitoring.

A low level of nitrosamines in biologics?

Many biologic products have the potential for nitrosamine contamination from the formation of impurities such as NDMA, NDSRIs or excipient-related nitrosamines. Indeed, given the relative abundance of amine functionalities in biologic products, it may seem that the susceptibility of this therapeutic class to the presence of these genotoxins could be higher than for traditional pharmaceuticals.

However, this would be contrary to the currently held belief that nitrosamine risk factors for biologics are marginal. This position is supported by a number of important considerations including:
(i) Generally, the high water purity used for the manufacturing, formulation and storage of biologics minimizes this source of potential nitrosating agents such as nitrates.
(ii) Purification procedures, often based on molecular size, minimize the potential for low molecular mass impurities.
(iii) Biologics are usually processed and stored using conditions that do not favor nitrosamine formation.
(iv) The in *vivo* generation of potentially mutagenic species from a nitrosated biologic API is considered very unlikely.
(v)

(v) Paradoxically, while biologics often do contain multiple reactive sites for nitrosamine formation, their molar abundance and type transforms such products into scavengers – actually reducing the effects from potential nitrosating agents (11). The European Federation of Pharmaceutical Industries and Associations has released a comprehensive review of these factors (12).

What is the risk level?

Clearly, if the biologics manufacturing process requires use of a known nitrosating agent, a thorough risk assessment must be performed in the same manner as for any other therapeutic. Generally, however, while the potential for nitrosamine contamination in biologic products is relatively low, for the reasons described previously, there are certain risks that have been recognized for this product type:

(i) Today, there are many biologic therapeutics that combine synthetically derived elements with those from cellular biosynthesis.

Indeed, there are now examples of complex peptides and proteins, produced entirely through synthetic processes; erythropoietin is one such example (13).

In these circumstances, when performing a risk assessment, the chemically produced components and intermediates should be considered as having the same potential for nitrosamine impurities as pharmaceutical products, although this is often mitigated by various methods of purification.

(ii) Container closure systems incorporating components such as elastomeric vial stoppers are a well-documented potential source of nitrosamines (14).

Recently, reaction between nitrocellulose and an amine-containing printing primer (components of blister primary packaging material) has been found to create nitrosamines (15) which, under certain conditions, can transfer into the final product.

(iii) While many of the most commonly used excipients such as inorganic salts, sugars, polyols and surfactants are of very little concern in terms of nitrosamine impurities, others, such as the antioxidant polyethylenimine may actually inhibit nitrosamine formation (16).

Stabilizing agents such as proline, arginine and glutamate are certainly capable of reaction with nitrosating agents, but the products from most amino acids are not carcinogenic (17).

Nevertheless, similar to nitrites, the potential risk from the presence of a reactive amine present in trace amounts in any excipient type will depend on the formulation composition and should be appropriately evaluated.

Ongoing challenges posed by nitrosamines in biologic products.

Clearly, there can be no single approach to solving the nitrosamine challenge and, even for the same product, a variety of solutions may be considered appropriate. For existing products, a thorough risk assessment is essential to provide confidence in the manufacturing, transport, and storage processes and the quality of raw materials.

For new products, such a risk assessment provides a means of minimizing the potential for nitrosamine contamination by revising the manufacturing processes or even proactively making use of nitrosating inhibitors or scavengers such as propyl gallate, ascorbic acid or maltol (18).

If analytical evaluation is necessary, biologic products may pose some unique challenges. Investigation of possible contamination by nitrosamines such as NDMA may be complicated because of interference from excipients such as surfactants, commonly used for biologic product formulations.

In contrast to the majority of pharmaceutical products, biologics usually contain numerous sites for nitrosation, thereby potentially giving rise to multiple NDSRI products – a difficult position to investigate and define. However, the expertise and capabilities of SGS's biologics and nitrosamine teams mean they can provide highly effective solutions to these complex analytical challenges.

The assertion that biologic products are at low risk of nitrosamine or NDSRI contamination seems well founded, and to date there have been no market withdrawals for this product class due to nitrosamine-related issues.

However, the significant diversity in molecular type, manufacturing processes and supply chains used for biologic therapeutics create new potential routes for nitrosation. It is therefore critical that the industry maintains its vigilance vis-à-vis these harmful impurities.

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