Risk Assessment – Nitrosamines presence

1. Introduction

N-Nitrosamines (NAMS) are a class of organic compounds which are typically not commercially produced or added to products. Although, they can be found in air, water, foods, cosmetics, tobacco and packaging materials. NAMS are present as a by-product of precursors used in manufacturing (vulcanization) of rubber, pesticides, and tobacco products.

2. Literature sources

A nitrosamine is an amine substituted by a nitroso (NO) group by the reaction of a primary, secondary or tertiary amine with a nitrosating agent (ED 2012; Scanlan, 1983). A nitrosamine can be formed by direct combination of an amine and nitrous acid (i.e. endogenously formed from nitrites and acidic gastric juices) as well as from a reaction between nitrites and amine groups of certain proteins (i.e. frying bacon). Having a general structure of $R_2N\text{-}N{=}O$, the majority of commonly occurring N-nitrosamines investigated by the International Agency for Research on Cancer (IARC) have been classified as carcinogenic (Kuhne, 2018). The stability of nitrosamines varies basing on pH (less stable in acidic solutions) and exposure to light. It is generally accepted that nitrosamine samples and standards are stable up to 14 days (Kuhne, 2018; RoC, 14th ed).

In the context of pharmaceutical industry, as well as in food and food contact materials, NAMS are not intentionally added substances (NIAS).
In pharmaceutical field, they may arise from amines present in coatings or printing inks*[1]. Nitrocellulose also includes normally traces of NAMS*[2], deriving from nitration process and following side reactions. Another possible NAMS source is degradation of pigment/dyes used in printing inks, in presence of a nitrosating agent.
Moreover, in food domain, they can be formed from constituents of food or products which are naturally present (i.e. amines that are present in the protein of meat) or added during production (nitrates or nitrites as preservatives) (RoC, 14th ed.).
Other exposure for humans could potentially be through contact with synthetic rubber and elastomers (Kuhne, 2018) with the formation of nitrosamines influenced by elevated temperatures (frying food) or high acidity (pH).

3. Analytical chemistry

Due to their carcinogenicity and mutagenicity, the pharmaceutical industry considers them a “special case” compounds. The detection of nitrosamines (NAMS) can be problematic due to their thermal instability and reactivity. Three valid EPA methods are associated with testing for certain NAMS and/or PAAs. Test mixtures containing the EPA test compounds are commercially available. The EPA methods reference GC/MS and LC/MS/MS detection methods, while GC/MS is likely to be more readily available for testing labs. NAMS greatly differ in hydrophobic character, which causes resolution on traditional liquid chromatography (C18) columns difficult.

However, analysis by LC/MS/MS is attractive due to shorter analysis methods. Typical LC separation is optimal on polar or hydrophilic type columns.

While GC/TEA is considered the gold standard for NAMS detection, this is a specialized detector that many may not have. Dallinga et al. (2001) provides a comparison of GC/MS and GC/TEA showing GC/MS detection limits lower than GC/TEA, with a strong correlation between the detection methods. Additionally, the US FDA is currently using HS-GC/MS and GC/MS/MS methods for the analysis of nitrosamine impurities in pharmaceuticals (FDA, 2018 and 2019).

In addition to gas chromatography - Thermal Energy Analyzer (GC-TEA), the European Committee for Standardization (CEN) specified liquid chromatography-tandem mass spectrometry (LC/MS/MS) with atmospheric pressure chemical ionization (APCI) as standard methods for the detection of nitrosamines (Kuhne et al 2018; CEN 2013).

Most of the methods for extraction are destructive and higher temperature methods are used when the substrates of interest are rubber, i.e. baby bottle nipples, o-ring seals in oral inhalers. Care should be taken when analyzing for NAMS so as not to ***create*** NAMS in the process. A more appropriate method might be something closer to the EPA sampling and analysis from water by SPE purification using an ethanol migration simulant, etc. Modifications could be made for the application.

With a variety of detection methods available, analytical labs have the option to choose a method suitable for their individual equipment and capabilities or employ an outside testing services. The above mentioned methods describe and detail extraction and detection of various NAMS. Most referenced methods are targeted SIM (selected ion monitoring) or MRM (multiple reaction monitoring), depending on analytical instrumentation. Methods employ internal standards with spike recovery. Where untargeted analyses are desired, an MS scanning mode may be appropriate with care taken for sensitivity of detection.

4. Risk Assessment

Industrial Associations, even before the popping out of NAMS in medical field, have tackled this issue, as it was already known that such compounds were/can be present in raw materials or finished goods. In this sense some papers were already published in the past years and are in some cases publicly available/have been published on specialized press.
There are also examples where food control authorities have tested printed articles looking for NAMS or their precursors.

About Aluberg's preparation of raw materials/equipment and manufacturing process, there is no presence of acidic step conditions, high temperature treatment, use of solvent, presence of stressing manufacturing conditions etc. that may address NAMS formation, so a contamination in this sense can be excluded. As NAMS aren't constituents of the article and process cannot generate them, their presence can be excluded.

In order to prevent possible cross contamination from product based on Nitrocellulose, specific cleaning procedures are implemented, with a complete and accurate decontamination of the production equipment, when a change in manufacturing campaign implies Nitrocellulose containing products.

For this last case (products which include Nitrocellulose or Pigments in their composition), the chosen Risk Assessment has been based on the analytical evidence of absence of NAMS by migration testing on finished article.

In order to confirm the outcome of the qualitative Risk Assessment, an analytical determination has been conducted, in order to substantiate this affirmation.
Analytical accredited lab chosen by Aluberg has used method EN 12868:2002. N-Nitrosamines and nitrosatable substances are extracted from a saline solution. After concentration and, for nitrosatable substances, conversion, Nitrosamines are determined through GC coupled with a MS detector. All analytical results are below analytical method's detection limits.
For products in which Nitrocellulose is a part of the formulation, analyses have been conducted as well, even if NDMA, NDEA and NDBA are known to be present as impurities.

5. Conclusions

Within Aluberg manufacturing processes, the majority of them doesn't imply the use of substances or polymers able to generate NAMS. Anyhow, for due diligence, and for sake of consumers' safety, even if a cross contamination can be excluded, they have been tested for NAMS presence. In the few cases, where Nitrocellulose or Pigments are used in Aluberg products manufacturing, there is the possibility of presence of NAMS within the products. Also such products have been tested.
Outcome of the Risk Assessment, analytically confirmed, is that in products which don't include Nitrocellulose in their composition there is no presence of Nitrosamines.

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*[1] = in some cases they may come also from the drug, like in case of Sartans and their derivatives.
*[2] = typically NDMA, NDEA and NDBA.