NITROSAMINES IN FOOD: CHEMISTRY, HEALTH RISKS, REGULATIONS, AND TESTING

SPEX | NSI | HPS | CHIRON

Analytical Reference Materials & PT by ZeptoMetrix®

NITROSAMINES IN FOOD: CHEMISTRY, HEALTH RISKS, REGULATIONS, AND TESTING

1. EXECUTIVE SUMMARY

Many compounds are highlighted each year as potential health threats or environmental contaminants. Among these are nitrosamines, which have been recognized as contaminants but are now receiving increased attention from both legislators and the public. The presence of nitrosamines in food has sparked substantial interest in understanding their formation, health risks, and regulatory controls. The main concern regarding nitrosamine formation in food arises from the use of nitrites as preservatives and colorants, particularly in processed meats such as bacon, sausages, and hot dogs. Additionally, smoking and cooking processes that involve high temperatures can increase the formation of nitrosamines. Nitrosamines are a class of chemical compounds formed primarily through the nitrosation reaction, where nitrites (NO_2^-) react with amines ($R\text{-}NH_2$) under acidic conditions or elevated temperatures. These compounds are recognized for their carcinogenic properties, raising significant health concerns not only in relation to food, but also due to their presence in tobacco and pharmaceutical products. This white paper aims to provide a comprehensive analysis of nitrosamine formation, the health risks associated with their consumption, the regulations that control their presence in food and beverage, and testing.

2. HISTORY OF NITROSAMINES

The discovery of nitrosamines dates back to the 1950s, when N-nitrosodimethylamine (NDMA) was identified as a potent carcinogen by British scientists, John Barnes, and Peter Magee. In their studies they found that 90% of the three hundred nitrosamines tested were carcinogenic. Further research revealed that nitrosamines were present in tobacco smoke, leading to the recognition of their role in lung cancer and other respiratory cancers.

In the years that followed starting in the 1970's, studies showed that nitrosamines could form in foods that were preserved with nitrates and nitrites. These compounds were found in processed meats, cheese, and beer, particularly in those that were smoked or cooked at high temperatures. Over the past few decades, there has been a significant increase in research studies focused on nitrosamines in food products (Figure 1).

Studies of Nitrosamines in Food by Decade*

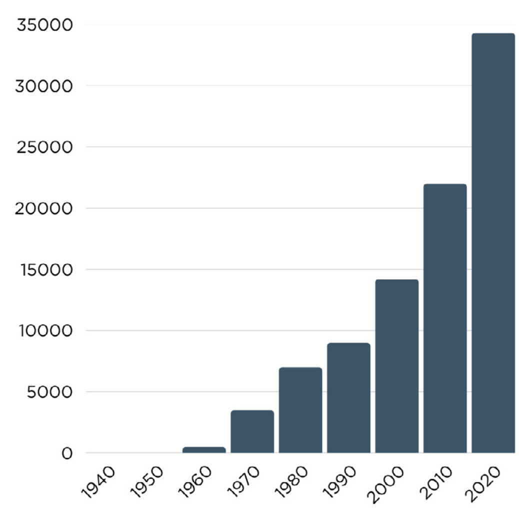

Figure 1. Number of Scientific Publications on Nitrosamines in Food by decade from 1960. *Current decade extrapolated based on publications through 2024.

Other studies found nitrosamines in pharmaceutical, nutraceutical, and tobacco products and they were identified as environmental targets from industrial activities. The largest categories of products or samples affected by nitrosamine contamination or byproducts are tobacco products (37.4%) followed by food products (24.2%) (Figure 2).

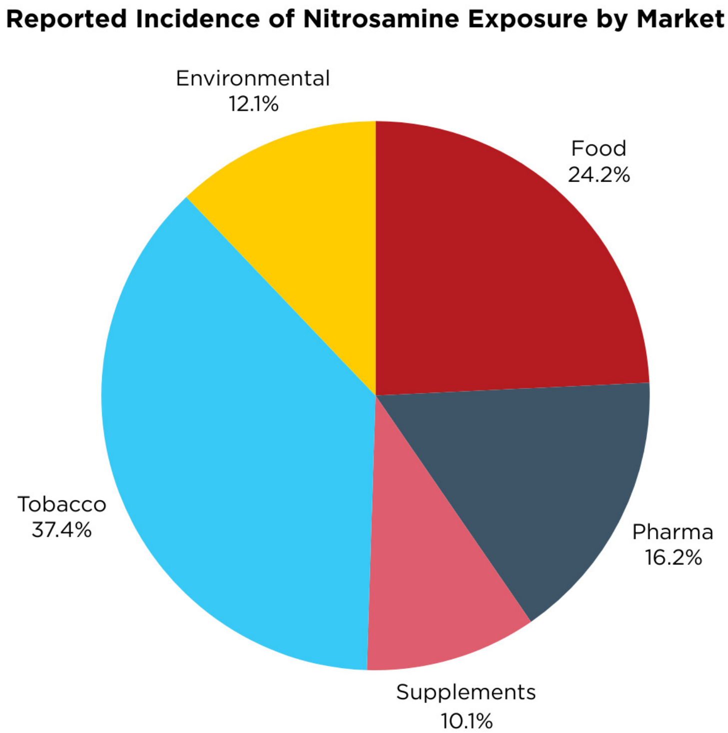

Figure 2. Incidence of Nitrosamine by Product Group or Market

Since the early discoveries, regulatory bodies such as the World Health Organization (WHO) and the International Agency for Research on Cancer (IARC) have classified nitrosamines as carcinogenic. These organizations have since implemented guidelines to control the levels of nitrosamines in food products, particularly those prone to nitrosation reactions involving nitrites and amines.

3. FORMATION OF NITROSAMINES IN FOOD

Nitrosamines can form in food through a chemical reaction between nitrites (NO_2^-) and amines ($R\text{-}NH_2$) (Figure 3). Nitrites are commonly added to processed meats as preservatives, while amines occur naturally in protein-rich foods. Under acidic conditions or high temperatures, nitrites and amines react (nitrosation reaction), leading to the formation of nitrosamines.

Nitrosation reaction is a process likely to occur in environments such as the human stomach (acidic) or during cooking involving high heat like frying, grilling or smoking. Nitrosamines are also a result of the reduction of nitrate (NO_3^-) to nitrite (NO_2^-) by some bacteria or heat, followed by nitrosation reaction. This occurs in foods preserved by nitrates, such as cured meats.

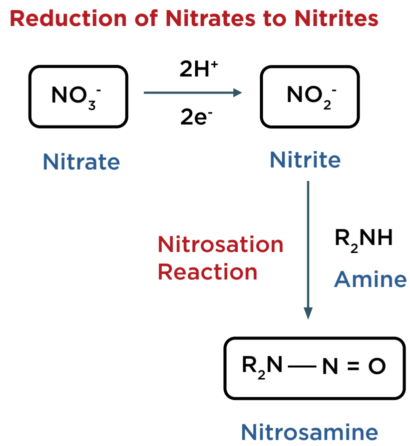

Figure 3: This diagram illustrates the chemical pathways leading to nitrosamine formation. In the two-step process, nitrates (NO_3^-) are first reduced to nitrites (NO_2^-), which then react with amines to form nitrosamines. Alternatively, in the one-step process, existing nitrites directly react with amines under acidic or high-temperature conditions, also resulting in nitrosamine formation.

The nitrosation reaction is common in processed meats such as bacon and sausages that undergo nitrite curing. The top products which contain nitrosamines are bacon, sausages, hot dogs, smoked, grilled, and cured meats and seafood, cheese, and beer (Figure 4).

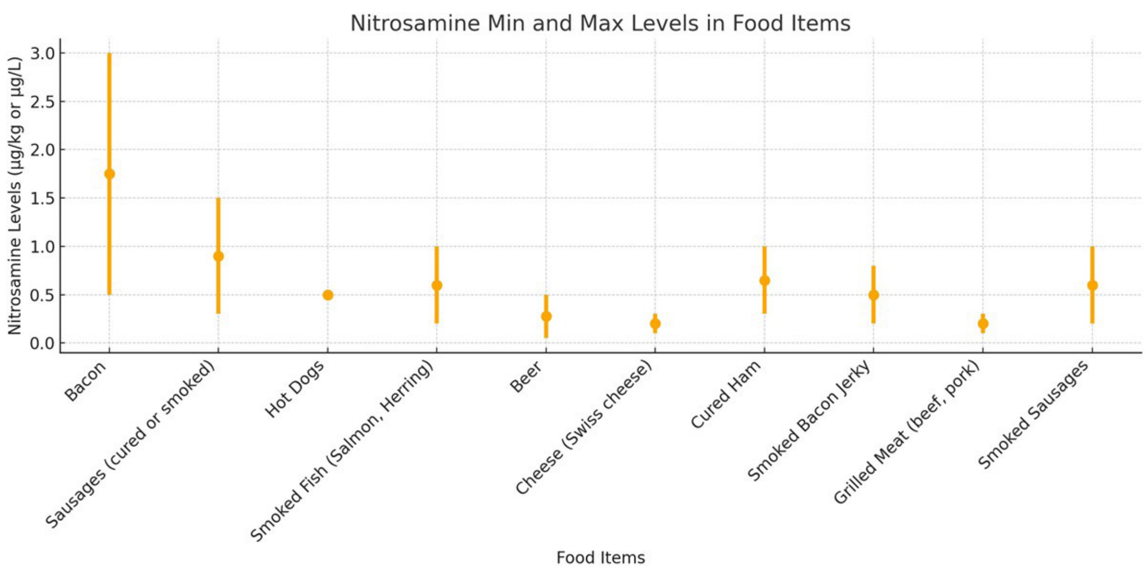

Figure 4. The reported incidence of nitrosamine levels in commonly cited foods high in nitrosamines.

3.1 Nitrosamine Formation by Microorganisms

Another route for nitrosamines formation is by bacterial nitrate reduction. Certain food and gut bacteria reduce dietary nitrates to nitrites. Some of the bacteria species involved include: Enterobacteriaceae species (e.g., E. coli, Salmonella), Clostridium species (e.g., C. perfringens, C. botulinum), Lactobacillus species (e.g., L. reuteri, L. plantarum) and Streptococcus species (e.g., S. mutans). The resulting nitrites can react with dietary or endogenous amines in the gastrointestinal tract to form nitrosamines, increasing the risk of cancer and genotoxicity. Table 1 summarizes bacterial species that can be found in food, the corresponding regulations and health limits.

Table 1. Common Bacterial Species from Food and Regulations

Bacterial Species	Product	Regulations/Concerns	Health Limits
Streptococcus spp.	Dairy, meat, beer	EPA monitors water for bacterial contamination, indirectly related to nitrosamines in water.	96 ng/day (FDA limit) for daily intake from food.
Clostridium spp.	Meat, cheese, dairy products, fermented foods	Known for producing nitrosamines in anaerobic conditions; regulated by FDA for food safety.	No specific limits; monitored in food and water systems.
Enterobacteriaceae	Meat products (e.g., beef, pork, dairy, fish)	No specific regulations; monitored in food safety and water quality standards.	No established limits, but levels in tobacco and food monitored.
Pseudomonas spp.	Fish, meat, dairy	Monitored in water and food safety regulations.	<0.1 µg/kg in some processed foods.
Salmonella spp.	Poultry, eggs, meat products	Regulated by FDA and USDA for food safety, including bacterial contamination.	No established limits, but regulated in food and beverages.
Vibrio spp.	Seafood, fish	FDA regulates seafood for pathogen and nitrosamine content.	No established limits in food, but regulated in tobacco.
Lactobacillus spp.	Cheese, yogurt, fermented vegetables, beer	Regulated by FDA for fermented food safety; minimal regulation for nitrosamines.	Monitored in smoked foods and water; limit varies by region.
Bacillus spp.	Meat, dairy, grains	Regulatory oversight for foodborne pathogens but not directly for nitrosamine production.	No established limits, but regulated in products containing amines.
Escherichia coli	Meat products, dairy, water	FDA and EPA regulate E. coli in food and water; indirectly related to nitrosamines.	FDA and EPA regulate E. coli in food and water; indirectly related to nitrosamines.

4. NITROSAMINES IN OTHER CONSUMER PRODUCTS

Nitrosamines are not only found in food but are also produced as byproducts from various industrial processes, including the tobacco industry, rubber manufacturing, and pharmaceuticals. Cigarettes, cigars, and smokeless tobacco products contain high levels of nitrosamines, especially NNK (N-nitrosonicotine) and NNN (N-nitrosonornicotine), both of which are potent carcinogens. Nitrosamines are produced during the vulcanization of rubber, where nitrosating agents, used as accelerators in the rubber-making process, react with amines. Some pharmaceuticals have been found to contain nitrosamine impurities as byproducts of chemical synthesis or storage. Despite their carcinogenicity, nitrosamines have been used in industrial processes and unintentionally formed in food products, posing a health risk to consumers.

5. HEALTH RISKS OF NITROSAMINES

Nitrosamines are widely recognized as carcinogenic and genotoxic, meaning they can cause DNA damage and increase the risk of developing cancer. NDMA, NNK, and NDEA are particularly dangerous nitrosamines found in tobacco and processed meats. Key health risks include several types of cancer as described in table 2.

Table 2. Nitrosamines in Food and their Health Risks

Name	Formula	Carcinogenic Status	Type of Reactions Involved	Health Effects	Health Limits
N-Nitrosodimethylamine (NDMA)	$C_2H_6N_2O$	Group 1 Carcinogen (IARC)	Nitrosation of dimethylamine with nitrites in food processing	Liver cancer, stomach cancer, genotoxicity	96 ng/day (FDA limit) for daily intake from food

Name	Formula	Carcinogenic Status	Type of Reactions Involved	Health Effects	Health Limits
N-Nitrosodiethylamine (NDEA)	$C_4H_9N_2O$	Group 2A Carcinogen (IARC)	Nitrosation of diethylamine with nitrites	Liver cancer, bladder cancer, genotoxicity	No specific limits; monitored in food and water systems
N-Nitrosopyrrolidine (NPYR)	C_4H_9N	Group 2A Carcinogen (IARC)	Nitrosation of pyrrolidine (alkaloid in fish, tobacco)	Liver cancer, lung cancer, genotoxicity	No established limits, but levels in tobacco and food monitored
N-Nitrosobutylamine (NBA)	C_4H_9N	Group 2A Carcinogen (IARC)	Nitrosation of butylamine	Liver cancer, genotoxicity	<0.1 µg/kg in some processed foods
N-Nitrosomorpholine (NMOR)	C_4H_9NO	Group 2A Carcinogen (IARC)	Nitrosation of morpholine (in beer, cheese, processed meats)	Lung cancer, genotoxicity	No established limits, but regulated in food and beverages
N-Nitrosopiperidine (NPIP)	$C_6H_{11}N$	Group 2A Carcinogen (IARC)	Nitrosation of piperidine	Liver cancer, genotoxicity	No established limits in food, but regulated in tobacco
N-Nitrosodi-n-propylamine (NDPA)	$C_6H_{11}N_2O$	Group 2A Carcinogen (IARC)	Nitrosation of n-propylamine in smoked foods and tobacco	Liver cancer, pancreatic cancer, genotoxicity	Monitored in smoked foods and water; limit varies by region
N-Nitrosocyclohexylamine (NCHA)	$C_6H_{11}N_3O$	Group 2A Carcinogen (IARC)	Nitrosation of cyclohexylamine (in tobacco, beer, cheese)	Liver cancer, genotoxicity	No established limits, but regulated in products containing amines

Public health concerns around nitrosamine exposure in food products, particularly those involving nitrite curing in meats, have led to the establishment of regulatory limits and testing methods to mitigate exposure.

6. GLOBAL REGULATIONS - NITROSAMINES IN FOOD

Regulation of nitrosamines in food varies globally, but several countries and international organizations established guidelines, limits, or monitoring protocols due to the carcinogenic potential of these compounds. Table 3 provides an overview of the main regulations and corresponding nitrosamine limits.

Table 3. Regulatory Limits for Nitrosamines in Food and Water

Agency/Region	Regulatory Focus	Limits/Advisory Levels
FDA / USDA / EPA (USA)	Food, water, pharmaceuticals	- EPA: NDMA health advisory: 0.07 µg/L in drinking water
		- FDA: Case-by-case (e.g., cured meats, recalls)
		- USDA: Nitrite limits + Vitamin C to inhibit nitrosamines
EFSA / European Commission (EU)	Food additives, contaminants	- EFSA risk assessment: lifetime cancer risk considered at exposure > 0.1 µg/kg bw/day NDMA
		- Cured meats: Nitrite max 150 mg/kg (with inhibitors to reduce nitrosamines)
Health Canada / CFIA	Water, food contaminants	- NDMA guideline for water: 0.00004 mg/L (0.04 µg/L)
		- Monitors and limits nitrites in processed meats
MHLW (Japan)	Food contaminants, additives	- Regulates nitrite levels in food (e.g., cured meat max: 70 mg/kg as sodium nitrite)
		- Requires inhibitors to reduce nitrosamine formation
National Food Safety Standards (GB – China)	Food additives, contaminants	- GB 2760-2014: Nitrite limit in meat ≤ 30–70 mg/kg, depending on product
		- Some standards address nitrosamine prevention, especially in preserved foods

7. TESTING METHODS FOR NITROSAMINES

Testing for nitrosamines in food and beverage involves specific and sensitive analytical techniques due to the low levels at which these compounds are typically present. The key methods in the US, EU, Canada, Japan and China include gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), and thermal energy analyzer (TEA). Different regulations or analytical methods call for different instruments.

The most common nitrosamines tested in food and beverage are:

N-Nitrosodimethylamine (NDMA)

- Commonly found in: Processed meats (like bacon, sausages), beer, and some dairy products.
- Concerns: NDMA is one of the most studied nitrosamines due to its carcinogenic potential. It is often formed during the processing or cooking of foods containing nitrates or nitrites.
- Regulation: There are stringent guidelines for NDMA levels in drinking water and food. The World Health Organization (WHO) has classified it as a probable human carcinogen.

N-Nitrosodiethylamine (NDEA)

- Commonly found in: Processed meats, beer, tobacco, and some fish.
- Concerns: Like NDMA, NDEA is a potent carcinogen and is typically tested in foods that have undergone curing or smoking processes.
- Regulation: NDEA is regulated in various countries, and its levels in food are monitored by agencies such as the U.S. Food and Drug Administration (FDA) and European Food Safety Authority (EFSA).

N-Nitrosopyrrolidine (NPYR)

- Commonly found in: Some beverages, particularly alcoholic drinks like beer and whiskey, and in tobacco products.
- Concerns: NPYR has been identified as carcinogenic, and while it is less common than NDMA and NDEA, it is still a concern in certain food and beverage products.
- Regulation: As with other nitrosamines, there are established limits for its presence in food and beverages.

N-Nitrosobutylamine (NBA)

- Commonly found in: Processed meats and beer.
- Concerns: NBA is also a potential carcinogen, and its levels are tested in food products, particularly those exposed to high levels of heat or preservation processes that involve nitrates and nitrites.
- Regulation: NBA is not as commonly found as NDMA or NDEA, but it is still subject to regulations in food safety standards.

N-Nitrosomorpholine (NMOR)

- Commonly found in: Processed meats and certain cheeses.
- Concerns: NMOR has shown carcinogenic properties in laboratory animals, and its presence is monitored in foods to reduce cancer risks.
- Regulation: Like other nitrosamines, NMOR is regulated to ensure it does not exceed safe levels in food products.

N-Nitrosodibutylamine (NDBA)

- Commonly found in: Processed meats, especially those that are smoked or cured.
- Concerns: This nitrosamine is less common than NDMA but still poses cancer risks. It is particularly a concern for products like sausages, hot dogs, and smoked fish.
- Regulation: Testing for NDBA is part of food safety procedures to ensure that levels stay within permissible limits.

7.1 Use of Certified Reference Materials (CRMs) in Nitrosamine Testing

Certified Reference Materials (CRMs) are fundamental to the accurate detection, identification, and quantification of nitrosamines in food products. Given the very low concentration at which nitrosamines can pose health risks—often in the nanogram per gram (ng/g) or nanogram per liter (ng/L) range—high-precision testing is essential. CRMs provide the benchmark needed to ensure the reliability, repeatability, and comparability of analytical results across laboratories and testing methods.

CRMs support method development, validation, and quality control, helping laboratories to meet stringent regulatory requirements set by food safety authorities and international standards bodies. They ensure traceability and come with well-documented certificates of analysis, which include uncertainty, stability data, and composition details.

By using nitrosamine CRMs, food testing laboratories can:

- Validate methods such as GC-MS, LC-MS/MS, and GC-HRMS
- Calibrate instruments with known concentrations
- Verify performance through quality control samples
- Demonstrate compliance with regulatory limits
- Reduce variability in interlaboratory studies

Ultimately, the use of CRMs is critical to ensuring the scientific integrity of nitrosamine testing and to protecting public health through trustworthy surveillance of food products.

Based on regulated methods, our brands Chiron and Spex offer nitrosamine CRMs as single-component solutions and multi-component mixes. Our portfolio includes a range of catalogue products, as well as customizable options to meet specific analytical needs (Table 4 and 5).

Table 4. Nitrosamines Singles

Part Number	Product Name	Synonym	CAS No.	Concentration	Matrix
CRM9004.2-K-ME	N-Nitrosodimethylamine	NDMA	62-75-9	1000 µg/mL	methanol
CRM9005.3-K-ME	N-Nitrosomethylethylamine	NMEA; NEMA	10595-95-6	1000 µg/mL	methanol
CRM9006.4-K-ME	N-Nitrosodiethylamine	NDEA	55-18-5	1000 µg/mL	methanol
CRM9007.6-K-ME	N-Nitrosodi-n-propylamine	NDPA	621-64-7	1000 µg/mL	methanol
CRM9008.8-K-ME	N-Nitrosodi-n-butylamine	NDBA	924-16-3	1000 µg/mL	methanol
CRM9009.4-K-ME	N-Nitrosopyrrolidine	NPYR	930-55-2	1000 µg/mL	methanol
CRM9010.5-K-ME	N-Nitrosopiperidine	NPIP	100-75-4	1000 µg/mL	methanol
CRM11210.3-K-ME	N-Methyl(nitrosoamino)ethanol	n/a	26921-68-6	1000 µg/mL	methanol
CRM14258.5-K-ME	Ethylisopropylnitrosamine	NEIPA, NIPEA	16339-04-1	1000 µg/mL	methanol
CRM14215.5-K-ME	4-(Methylnitrosoamino)butanoic acid	NMBA	61445-55-4	1000 µg/mL	methanol
CRM8986.4-K-AN	N-Nitrosopiperazine	1-Nitrosopiperazine	5632-47-3	1000 µg/mL	acetonitrile
CRM9018.7-K-ME	N-Nitrosomethylphenylamine	NMPA	614-00-6	1000 µg/mL	methanol
CRM9019.4-100-AN	1,4-Dinitrosopiperazine	n/a	140-79-4	100 µg/mL	acetonitrile
CRM9326.4-K-ME	N-Nitrosomorpholine	NMOR	59-89-2	1000 µg/mL	methanol
CRM9328.12-K-ME	N-Nitrosodiphenylamine	Ndiphen	86-30-6	1000 µg/mL	methanol
CRM9329.14-K-ME	N-Nitrosodibenzylamine	NDBzA	5336-53-8	1000 µg/mL	methanol
CRM10095.6-K-ME	N-Nitrosodiisopropylamine	NDIPA	601-77-4	1000 µg/mL	methanol
CRM9666.4-K-ME	N-Nitrosodiethanolamine	NDELA	1116-54-7	1000 µg/mL	methanol

Table 5. Nitrosamines Mixes

Part Number	Product Name	Total Volume	Solvent
521-A	EPA Method 521 Nitrosamine Native Mix	1 mL	Methylene Chloride

8. CONCLUSION

Nitrosamines in food represent a significant health risk due to their carcinogenic and genotoxic properties. These compounds are primarily formed through nitrosation reactions, often triggered by the use of nitrite preservatives in processed meats, beer, and cheese. Regulatory bodies like the FDA, EPA, EU, and WHO have established limits for nitrosamine levels in food and beverage, and testing methods have been employed to monitor and ensure compliance with safety standards. Ongoing surveillance and mitigation efforts remain essential for minimizing consumer exposure to nitrosamines.

9. REFERENCES

1. International Agency for Research on Cancer (IARC). (2018). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 100F. Nitrosamines. Available from: https://monographs.iarc.fr/ENG/Monographs/vol100F/

2. World Health Organization (WHO). (2002). Nitrosamines in Food. WHO Food Additives Series 50. Available from: https://www.who.int

3. National Toxicology Program (NTP). (2011). Report on Carcinogens. 12th Edition. U.S. Department of Health and Human Services. Available from: https://ntp.niehs.nih.gov/

4. Boffetta, P., & Jourenkova, N. (1997). N-Nitroso Compounds and Cancer. Environmental Health Perspectives, 105(Suppl 6), 1129–1139. doi:10.1289/ehp.97105s61129

5. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. (2010). Monograph on the Evaluation of Carcinogenic Risks to Humans. N-Nitroso Compounds. IARC Press. ISBN: 9789283201574.

6. Patocka, J., & Kuca, K. (2019). Nitrosamines: Chemical and Toxicological Aspects. Environmental Toxicology and Pharmacology, 67, 103–112. doi:10.1016/j.etap.2019.02.007

7. Morgenstern, J., & Ross, P. (2017). Bacterial Influence on Nitrosamine Formation. Journal of Food Safety, 37(3), 42–53. doi:10.1111/jfs.12349

8. Fujimura, Y., & Nakajima, H. (1993). Bacterial Nitrosation and N-Nitroso Compounds in Foods. Microbial Ecology in Health and Disease, 6(2), 101–108. doi:10.3109/08910609309142078

9. Miller, M. J., & Geddy, A. C. (2013). Bacteria and Nitrosamine Formation: Implications for Food Safety and Water Quality. Environmental Microbiology Reviews, 8(2), 195–211. doi:10.1016/j.envmic.2012.07.008

10. Loftus, T. D., & Dreher, G. A. (2006). Bacterial Influence on Nitrosamine Formation in Food. Food Control, 17(8), 673–681. doi:10.1016/j.foodcont.2005.06.021

11. FDA Guidance for Industry: Control of Nitrosamine Impurities in Human Drugs. (2021). U.S. Food and Drug Administration. FDA Guidance Document. FDA Acceptable Intake Limits.

12. EMA Guidance for Marketing Authorisation Holders: Nitrosamine Impurities. (2021). European Medicines Agency. Article 5(3) of Regulation (EC) No 726/2004.

13. Health Canada Guidance Document: Nitrosamine Impurities in Medications. (2021). Health Canada Guidance.

14. PMDA Guidance on Nitrosamine Impurities. (2021). Pharmaceuticals and Medical Devices Agency (PMDA), Japan. Overview.

15. NMPA Guidance on Control of Nitrosamine Impurities. (2021). National Medical Products Administration (NMPA), China. Guidance.

16. MFDS Guidance on Nitrosamine Impurities. (2021). Ministry of Food and Drug Safety (MFDS), South Korea. Guidance.

17. TGA Guidance on Nitrosamine Impurities. (2021). Therapeutic Goods Administration (TGA), Australia. Guidance.

18. ANVISA Resolution RDC No. 53/2015. (2015). National Health Surveillance Agency (ANVISA), Brazil. Resolution.

19. CDSCO Guidance on Nitrosamine Impurities. (2021). Central Drugs Standard Control Organization, India. Guidance.

20. SAHPRA Guidance on Nitrosamine Impurities. (2021). South African Health Products Regulatory Authority. Guidance.

21. USP General Chapter <1469> Nitrosamine Impurities. (2021). United States Pharmacopeia (USP). General Chapter <1469>.

22. EPA Method 607: Nitrosamines. (2016). U.S. Environmental Protection Agency (EPA). Method 607.

23. AOAC Official Method 982.14. (1982). Official Methods of Analysis, Association of Official Agricultural Chemists. Method 982.14.

24. ASTM D4767-02. (2002). ASTM International. Standard Test Method for Determining Nitrosamines in Gasoline. ASTM D4767-02.

LEARN MORE & CONTACT

The Americas
1.800.274.5487
analytical.cs@zeptometrix.com