DOI 10.1002/mnfr.201400286 *Mol. Nutr. Food Res.* 2015, *59*, 106–128

REVIEW

Nitrate and nitrite in the diet: How to assess their benefit and risk for human health

Michael Habermeyer[1], Angelika Roth[1], Sabine Guth[1], Patrick Diel[2], Karl-Heinz Engel[3], Bernd Epe[4], Peter Fürst[5], Volker Heinz[6], Hans-Ulrich Humpf[7], Hans-Georg Joost[8], Dietrich Knorr[9], Theo de Kok[10], Sabine Kulling[11], Alfonso Lampen[12], Doris Marko[13], Gerhard Rechkemmer[11], Ivonne Rietjens[14], Richard H. Stadler[15], Stefan Vieths[16], Rudi Vogel[17], Pablo Steinberg[18] and Gerhard Eisenbrand[1]*

[1] Department of Food Chemistry and Toxicology, University of Kaiserslautern, Kaiserslautern, Germany**

Received: April 29, 2014
Revised: July 29, 2014
Accepted: August 4, 2014

Nitrate is a natural constituent of the human diet and an approved food additive. It can be partially converted to nitrogen monoxide, which induces vasodilation and thereby decreases blood pressure. This effect is associated with a reduced risk regarding cardiovascular disease, myocardial infarction, and stroke. Moreover, dietary nitrate has been associated with beneficial effects in patients with gastric ulcer, renal failure, or metabolic syndrome. Recent studies indicate that such beneficial health effects due to dietary nitrate may be achievable at intake levels resulting from the daily consumption of nitrate-rich vegetables. *N*-nitroso compounds are endogenously formed in humans. However, their relevance for human health has not been adequately explored up to now. Nitrate and nitrite are per se not carcinogenic, but under conditions that result in endogenous nitrosation, it cannot be excluded that ingested nitrate and nitrite may lead to an increased cancer risk and may probably be carcinogenic to humans. In this review, the known beneficial and detrimental health effects related to dietary nitrate/nitrite intake are described and the identified gaps in knowledge as well as the research needs required to perform a reliable benefit/risk assessment in terms of long-term human health consequences due to dietary nitrate/nitrite intake are presented.

Keywords:
Benefit/risk analysis / Biomarkers / Nitrate / Nitrite / *N*-nitroso compounds

Additional supporting information may be found in the online version of this article at the publisher's web-site

1 Introduction

The Senate Commission on Food Safety (SKLM) of the German Research Foundation (DFG) has repeatedly addressed the potential health risks due to nitrosamines, nitrate, and nitrite in connection with endogenous nitrosation reactions. In this context, gaps in knowledge and research needs were last summarized by the SKLM in 1994 [1]. In view of

Correspondence: Professor Gerhard Eisenbrand, Department of Chemistry, Food Chemistry and Toxicology, University of Kaiserslautern, Erwin-Schroedinger-Straße 52, D-67663 Kaiserslautern, Germany
E-mail: sklm@tiho-hannover.de
Fax: +49-631-205-3085

Abbreviations: **7-CEG**, 7-carboxyethylguanine; **ADI**, acceptable daily intake; **AP**, amidopyrine; **ATNCs**, apparent total nitroso compounds; **BMDL10**, bench mark dose lower confidence limit 10%; **b.w.**, body weight; **CVDs**, cardiovascular diseases; **CYP 450**, cytochrome P450; **DHU**, 5,6-dihydrouracil; **EFSA**, European Food Safety Authority; **HNO_2**, nitrous acid; **IARC**, International Agency for Research on Cancer; **JECFA**, Joint FAO/WHO Expert Committee on Food Additives; **MOE**, margin of exposure; **NDEA**, *N*-nitrosodiethylamine; **NDMA**, *N*-nitrosodimethylamine; **NNK**, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; **NNN**, *N*-nitrosonornicotine; **NO**, nitrogen monoxide; **N_2O_3**, dinitrogen trioxide; **N_2O_4**, dinitrogen tetroxide; **NOC**, *N*-nitroso compounds; **NOS**, NO synthases; **eNOS**, endothelial NOS; **NPRO**, *N*-nitroso products of 3-hydroxyproline and proline; **O^6CMdG**, O^6-carboxymethyl-2′-deoxyguanosine; **O^6MedG**, O^6-methyl-2′-deoxyguanosine; **O^6CMG**, O^6-carboxymethylguanine; **SCCS**, Scientific Committee on Consumer Safety; **SKLM**, Senate Commission on Food Safety; **SCF**, Scientific Committee on Food; **SUMO**, small ubiquitin-related modifiers; **WCRF/AICR**, World Cancer Research Fund/American Institute for Cancer Research; **XOR**, xanthine oxidoreductase

*Additional corresponding author: Professor Pablo Steinberg, E-mail: Pablo.Steinberg@tiho-hannover.de
**For other affiliations please see Addendum

the controversial discussion concerning potential detrimental versus beneficial health effects related to dietary nitrate and nitrite intake, the SKLM organized a round table meeting on "Nitrate and nitrite in the diet, benefit/risk for human health" with experts from The Netherlands, Sweden, UK, USA, and Germany in Bonn, Germany, on November 27, 2012. Following its mandate for the evaluation and advice concerning the effects of food and food constituents on human health, the SKLM reviewed the evidence regarding the beneficial and detrimental health effects related to dietary nitrate/nitrite intake, identified gaps in knowledge, and highlighted research needs to perform a reliable benefit/risk assessment in terms of long-term human health consequences due to dietary nitrate/nitrite intake. This information is now presented in form of a review, and extended abstracts of the presentations given at the above-mentioned meeting are available as electronic Supporting information to this review.

An increased consumption of vegetables is widely recommended because of their generally recognized beneficial health effects. In 2007, the World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) rated the evidence as "convincing to probable" that diets high in vegetables and/or fruits protect against cancers of the mouth and pharynx, esophagus, lung, stomach, colon and rectum, larynx, pancreas, breast, and bladder [2]. It is not clear at present whether such beneficial health effects associated with high vegetable intake become evident because or in spite of a concomitantly high exposure to dietary nitrate. Recent evidence from experimental and human intervention studies as well as epidemiological observations suggest beneficial health effects including, for instance, effects on blood pressure, myocardial infarction, or stroke associated with enhanced dietary nitrate intake [3–7]. However, nitrate from exogenous sources has also given rise to health concerns because of the potential endogenous formation of *N*-nitroso compounds (NOC). It should be mentioned that exposure to nitrate or nitrite may also be associated with the formation of methemoglobin in blood, the so-called methemoglobinemia, an adverse health effect especially in infants [8]. However, methemoglobinemia will not be considered in the present review.

1.1 Exposure and former risk assessment of nitrate and nitrite

Nitrate is a naturally occurring or chemically synthesized compound that forms part of the nitrogen cycle. It is a natural constituent as well as an approved food additive. In general, uptake of nitrate from exogenous sources in humans predominantly results from the consumption of nitrate-rich foods. In addition to dietary uptake, endogenous nitrogen monoxide (NO) formation from arginine is considered to be another major contributor to the overall internal exposure of humans to NO, nitrite, nitrate, and several nitrosyl intermediates. A major part of dietary nitrate exposure (about 50–70%) is due to the consumption of vegetables, thereby reflecting their often substantial nitrate contents [9]. In view of the large variation in the median concentrations of nitrate in different vegetables (e.g., from 1 mg/kg in peas and Brussels sprouts to 4800 mg/kg in rocket (*Eruca sativa* and *Diplotaxis tenuifolia*)), the European Food Safety Authority (EFSA) considered different scenarios for nitrate exposure estimations, assessing for adults a mean dietary nitrate uptake of 157 mg/day, equivalent to 2.6 mg/kg body weight (b.w.)/day based on a b.w. of 60 kg [9]. An acceptable daily intake (ADI) for nitrate was deduced by the Scientific Committee on Food (SCF) in 1990 [10] and confirmed in 1995 [11]. The no observed effect level of 370 mg nitrate/kg b.w./day was derived from long-term studies in rats and a subchronic toxicity study in dogs. Applying an uncertainty factor of 100 an ADI of 222 mg/day (0–3.7 mg/kg b.w./day) for nitrate was calculated. The ADI was confirmed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2002 [12]. The CONTAM Panel of EFSA concluded that in the absence of significant new toxicological and toxicokinetic data, there was no need to reconsider this ADI [9]. In its nitrate uptake assessment, the CONTAM panel of EFSA also noted that (i) individual consumption habits appear to be of great importance; (ii) the nitrate uptake of people consuming high amounts of nitrate-rich vegetables such as lettuce, spinach, rocket, beets, and radish may be considerably higher than the mean value and may thereby exceed the current ADI.

Nitrite is formed naturally at rather low steady-state concentrations in the nitrogen cycle by nitrogen fixation and is subsequently converted to nitrate, a major nutrient assimilated by plants. Consumer exposure to nitrite results as a consequence of its use as a food preservative and, to a lesser extent, from its presence in certain vegetables [9]. Assuming a mean nitrite concentration of 0.5 mg/kg for all vegetables, as reported in the United Kingdom's 1997 Total Diet Study, a recommended consumption of 400 g vegetables per day would result in a dietary exposure of 0.2–0.8 mg nitrite/day, equivalent to 3–13 μg/kg b.w./day based on a b.w. of 60 kg [9]. A further publication by EFSA on nitrite in meat products reported mean dietary exposure levels of adults to nitrite in the range of 0.04–0.23 mg/kg b.w./day (by combining nationwide data on food consumption with the maximum permitted usage levels for the additive). Based on the reported average nitrite levels, a consumer exposure of 5–30 μg/kg b.w./day was estimated [13]. In 2002, JECFA set an ADI of 0–0.07 mg/kg b.w. for nitrite, based on its heart and lung toxicity in a long-term study of the U.S. National Toxicology Program (NTP) in rats and a safety factor of 100 [14].

2 Metabolic fate and turnover of NO, nitrite, and nitrate in the organism

In the organism, nitrate and nitrite may function as an alternative source for NO, an important and multifaceted physiological signaling molecule, normally generated from arginine

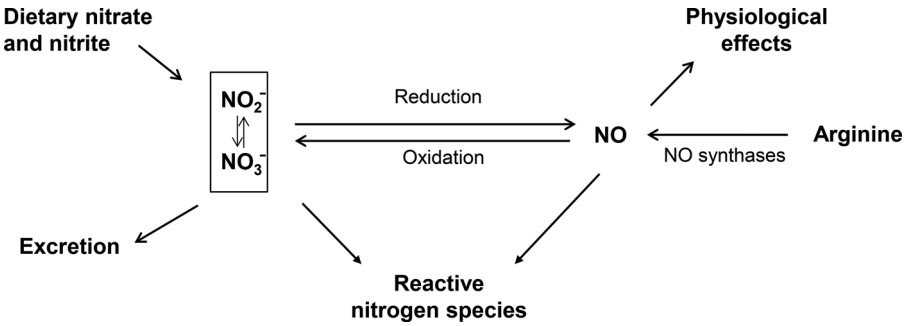

Figure 1. Metabolic fate of nitrate, nitrite, and NO.

by NO synthases (NOS; see below). Although NO is rather short-lived, it may react under oxidative conditions, i.e., in the presence of oxygen and/or reactive oxygen species, to give rise to nitrite, nitrate as well as nitrosyl peroxide and/or corresponding radical/ionic intermediates contributing to oxidative/nitrosative damage. These NO-derived species may lead to an array of reaction products under cellular or in vivo conditions, including *N*-, *S*-, and *O*-nitroso compounds as well as nitro derivatives of amino acids, peptides, proteins, and DNA bases [15–20]. The biological activities of such NO-related secondary products have not been fully explored up to now. They may include pharmacological effects, e.g., on blood vessels and blood pressure, the induction of oxidative stress/inflammation and ultimately the endogenous formation of NOC.

Nitrate biosynthesis in humans was first described more than 35 years ago [21, 22]. Early on, nitrite and nitrate, whether endogenously synthesized or taken up from exogenous sources, were taken into consideration as an alternative source for endogenous NO [23–28]. NO can be endogenously oxidized to nitrate and nitrite [28–30] and the latter can in part undergo reduction and cycling back to bioactive NO in blood and tissues before terminal excretion, e.g., as urinary nitrate [31] (Fig. 1). The biological messenger NO is commonly generated by oxygen-dependent NOS from L-arginine [28, 32]. The NOS family comprises inducible NOS as well as constitutive NOS, like endothelial (eNOS) and neuronal NOS [33].

Inflammation has been found to influence the metabolism of nitrate and nitrite. Infections induced by bacteria, parasites, or viruses as well as inflammatory diseases such as gastritis, hepatitis, and colitis have been recognized as risk factors for human cancers of the stomach, liver, and colorectum, respectively. Such inflammatory conditions have been shown to favor the enhanced biosynthesis of NO, nitrite, and nitrate [34, 35]. The Griess nitrite test, which is used to determine nitrite in urine, has a long history as diagnostic tool for the recognition of urinary tract infections [36]. An increase in blood nitrate was also shown to occur in lipopolysaccharide-treated mice or mice infected with *Mycobacterium bovis* and was reported to result from enhanced NO/nitrate biosynthesis via T-lymphocyte-mediated activation of macrophages [37]. It has been shown that inflammation in many tissues is accompanied by an upregulation of inducible NOS that is capable of producing NO in excess for a prolonged period of time [38, 39].

An increased endogenous formation of NOC has been shown to occur in humans infected with the Southeast Asian liver fluke (*Opisthorchis viverrini*) [40] and later confirmed experimentally in a study with hamsters [34]. *N*-nitrosothiazolidine 4-carboxylic acid was measured as a marker of endogenous nitrosation in the urine of hamsters, nitrosoproline (NPRO (*N*-nitroso products of 3-hydroxyproline and proline)) in humans. The infection of the bile ducts with the trematode enhances the risk of developing cholangiocellular carcinomas in humans [41]. Further examples are the urinary and the intestinal schistosomiasis, also known as bilharzia, caused by *Schistosoma haematobium* and *Schistosoma mansoni*, respectively. Infection with these trematodes is a risk factor for bladder and intestinal cancer [42], and in the urine of infected humans elevated concentrations of NOC were measured by gas chromatography [43–45]. Nitrite concentrations in the gastric fluid have been shown to be (directly) correlated with intragastric pH. Under conditions of insufficient production of gastric acid (hypo- or achlorhydria) bacterial contamination of gastric juice is common, and nitrate-reducing microorganisms are regularly found. Thus, formation of NOC under microbial catalysis in human gastric juice, urine, or by microorganisms from saliva samples has been shown [46–52]. Evidence has been accumulating that NO-derived reactive nitrogen species possess pathogenic potential, and it has been proposed that DNA and tissue damage induced by these reactive species may contribute to increased mutation rates, genome instability, apoptosis, and associated tissue regeneration, encompassing a proliferative response of cells [53–55]. Enhanced formation of 8-nitroguanosine and 3-nitrotyrosine has also been observed during microbial infection, and these compounds may be used as potential biomarkers for "nitrative stress" [53, 55].

Dietary nitrate is rapidly absorbed in the upper gastrointestinal tract and distributed via the blood circulation. It reaches the salivary glands and is actively transported from blood into saliva. The salivary nitrate level may be up to 20 times higher than the plasma level [48, 49, 56, 157]. The protein sialin has been proposed to play a role in nitrate transport [58]. In the oral cavity salivary nitrate can be reduced

to nitrite, predominantly by commensal bacteria [59]. The secreted nitrate as well as the nitrite generated in the oral cavity are swallowed, thereby reentering the gastrointestinal tract. Approximately 25% of nitrate originally ingested is secreted through the salivary glands, 6–7% of the total nitrate being converted to nitrite in the oral cavity during enterosalivary circulation [56, 60]. To some extent, saliva-derived nitrite may also contribute to NO formation under acidic conditions in the stomach. Exhalation of NO in the breathing air has been taken as an indication for such an intragastric NO formation from nitrite [61, 62]. The released NO has been discussed to be potentially beneficial due to antimicrobial effects, notably against *Helicobacter pylori* [63, 64]. However, it has also been reported that *H. pylori* exhibits a strong resistance against NO [65]. It has been proposed that this effect may be due to the low affinity of cytochrome c oxidase for this radical within the membrane/lipid bilayers of *H. pylori* [65] or to an unprecedented nitric oxide detoxifying system [66]. Some nitrite may survive gastric passage and enter the systemic circulation. Nitrite levels in plasma appear to be directly correlated to the intake of nitrate [23, 67, 68].

The nitrate–nitrite–NO pathway is believed to affect NO homeostasis. This may be relevant under circumstances when oxygen-dependent NOS become dysfunctional [68–71]. In blood and tissues, NO can be generated independently from NOS by a variety of enzymes and proteins acting as nitrite reductases, including, among others, flavoproteins and cytochrome P450 (CYP 450), deoxygenated hemoglobin, myoglobin, xanthine oxidase, and mitochondrial respiratory chain enzymes [24, 26, 69, 72–77].

3 Potential beneficial health effects of nitric oxide, nitrate, and nitrite

3.1 Physiological effects of NO

Since the discovery of endogenous NO formation, it became clear that NO is a pleiotropic signaling molecule relevant for a number of NO-mediated physiological effects. The radical NO easily diffuses across cell membranes and is capable of interacting with various receptor proteins. A key NO-related event is the coupling of a nitroso moiety from NO-derived metabolites to a reactive cysteine leading to the formation of *S*-nitrosothiols. Such NO-induced *S*-nitrosation processes take part in a multitude of signaling events in the cell, influencing many physiological functions. These comprise, among others, the regulation of blood pressure [78, 79] and blood flow by mediating vasodilation [80], the maintenance of blood vessel tonus [81], the inhibition of platelet adhesion and aggregation [82, 83], and neurotransmission [84–86]. Likewise, modulation of mitochondrial function and energetics by reactive NOs [87], modulation of the immune system, the endocrine system, and retina function [88–91] have been reported. In addition, the role of NO-related effects in liver regeneration [92], heart development [93], and diseases like cochlear function and hearing diseases [94], cluster headache [95], and cystic fibrosis [96] are under investigation.

NO also has been shown to induce apoptosis in macrophages and endothelial cells [97, 98]. A potential role of NO in carcinogenesis and tumor progression is largely unexplored. Depending on the concentration of NO and the tumor microenvironment, divergent effects may be expected [99, 100]. High cellular activity of NOS appears to be associated with cytostatic or cytotoxic effects on tumor cells [101]. In vitro studies with human cancer cells point to an inhibitory effect of nitrite on cancer cell replication mediated by NO [102].

Based on the observation that nitrate and/or nitrite can contribute to endogenous NO formation, dietary uptake of these compounds has been associated with NO-like physiological effects in humans and other mammals. For instance, this applies to blood pressure, vascular control, and vasodilation [27, 103–109]. Furthermore, protection against ischemia reperfusion injury in brain, heart, liver, and kidney [110–113], improvement of revascularization in chronic ischemia [114], decreasing leukocyte recruitment in microvascular inflammation [115], mobilization of angiogenic cells [116] as well as antiplatelet and antiaggregation effects in blood [117–119] have been reported. Moreover, attenuation of oxidative stress [120] as well as stimulation of mucosal blood flow and mucus formation in the gastrointestinal tract [121, 122] have been observed. This may contribute to the reported gastroprotective role of salivary nitrite, exemplified by studies indicating increased gastric mucosal blood flow and mucus thickness in rats.

In addition, dietary inorganic nitrate has been shown to alleviate features of the metabolic syndrome in eNOS-deficient mice [123]. The metabolic syndrome is a combination of physiological disorders that increase the risk of developing cardiovascular diseases (CVDs) and type-2 diabetes. Decreased synthesis of bioavailable NO by eNOS has been proposed to represent a central event in the development of the metabolic syndrome [124, 125].

3.2 Influence of nitrate and nitrite on the cardiovascular system

Potential protective mechanisms related to CVDs include vasodilation, inhibition of endothelial dysfunction, and inhibition of platelet aggregation. Endothelium-derived NO is an important signaling agent in the regulation of blood pressure [79]. NO-mediated regulation of vascular tone involves increased cyclic guanosine monophosphate formation and subsequent relaxation of vascular smooth muscle [78, 126].

It has been shown that relaxation of the rat aorta correlated with NO generation from nitrite in vitro. This relaxation could be prevented by an inhibitor of the NO-sensitive guanylyl cyclase, thereby confirming the involvement of the NO-mediated signaling pathway [127]. Local forearm blood

flow at rest und during exercise [106] was found to depend on nitrite reduction to NO by deoxyhemoglobin [73]. Nitrite in the vascular tissue has been proposed to function as a NO reservoir, enabling the release of physiologically relevant quantities of NO in cases of compromised NOS activity and independently of NOS [128]. Accordingly, hypertension in eNOS knockout mice was prevented by supplementation with dietary nitrate [123]. Nitrite is considered to be a major NO source under hypoxemic conditions, and under these conditions deoxyhemoglobin and xanthine oxidoreductase (XOR) may play a substantial role in NO formation in the blood vessel wall and in erythrocytes [129]. In a clinical study with a randomized double-blind crossover design, blood pressure decreased in healthy volunteers after supplementation with 0.1 mmol nitrate/kg b.w. for 3 days [105]. According to the authors, this would correspond to the consumption of about 150–250 g nitrate-rich vegetables such as spinach, lettuce, or beetroot.

The enterosalivary circulation of nitrate and its reduction to nitrite by the microbiota of the oral cavity influence plasma concentrations of nitrate and nitrite. Antiseptic mouthwash treatment was found to reduce the circulating nitrite concentration [57] and to correlate with an increase of systolic and diastolic blood pressure in rats and humans [130, 131].

Nitrite may also become an important alternative source for NO during ischemia and contribute to reducing myocardial ischemia-reperfusion damage. Enzyme-independent nitrate and/or nitrite reduction to NO in the ischemic heart influenced blood flow regulation and metabolic activity during hypoxia/ischemia and was proposed to mediate postischemic injury, depending on the duration of NO production [132, 133].

Homogenized human and rat myocardium was found to generate NO from nitrite under ischemic conditions. Moreover, reduction of infarct size in rat hearts in vitro via nitrite reduction to NO depended on XOR activity [111]. Cytoprotective effects of sodium nitrite during in vivo ischemia reperfusion were confirmed in the heart and liver of mice [134] and in the brain of rats [110]. The protective effects against ischemia/reperfusion injury in the kidney were shown to depend on XOR activity in rats [113]. In human studies, orally ingested nitrate was found to protect against endothelial ischemia-reperfusion injury [135, 136].

The role of nitric oxide and cyclic guanosine monophosphate in the inhibition of platelet adhesion to vascular endothelium [82] was shown almost simultaneously with the discovery of NO as the endothelium-derived relaxation factor [81, 137, 138]. Ingestion of potassium nitrate was shown to cause an increase in the formation of gastric *S*-nitrosothiols and an inhibition of platelet aggregation in humans [119]. The attenuation of platelet reactivity was found to be dependent on erythrocytes and deoxygenation; nitrite alone at physiological concentrations had no effect on platelet aggregation in plasma [139].

3.3 Beneficial health effects of dietary nitrate from foods

Consumption of fruits and vegetables, particularly of green leafy vegetables, appears to protect against coronary heart disease and ischemic stroke risk [140, 141]. In a study with two prospective cohorts, increased fruit and vegetable consumption was associated with modest benefits regarding the progression of CVDs [142]. The high nitrate content of some vegetables may explain some of the beneficial health effects of this food group, including the protection against CVD and type-2 diabetes [143, 144].

Beetroot juice, known to often contain high nitrate levels [9], was found to exhibit blood pressure lowering and vasoprotective effects [104]. The consumption of 250–500 mL beetroot juice per day led to a decrease in systolic blood pressure of 5.4–12 mmHg and to a decrease in diastolic blood pressure of up to 10 mmHg. Mean nitrate concentrations in the consumed beetroot juice were 22–45 mmol/L, corresponding to an intake of 341–1395 mg nitrate/person/day or 5.7–23.3 mg nitrate/kg b.w./day (154–630% of the ADI value) [145, 146]. Nitrate ingestion with beetroot juice was also reported to prevent endothelial dysfunction subsequent to an acute ischemic insult in the human forearm and to attenuate ex vivo platelet aggregation. Disruption of the enterosalivary circulation and interruption of the partial conversion of nitrate to nitrite (by spitting out all the saliva) blocked the decrease in blood pressure and abolished the inhibitory effects on platelet aggregation, thereby confirming the pivotal role of nitrite generated by the oral microbiota. In a meta-analysis of 16 studies (7–30 participants/study, duration 2 h to 14 days), beetroot juice supplementation as well as nitrate ingestion were associated with a significant reduction in systolic blood pressure. The daily amount of inorganic nitrate (sodium or potassium nitrate) consumed in these studies ranged from 2.5 to 24 mmol/dose, corresponding to 155–1488 mg nitrate (70–672% of the ADI value), and the amounts of inorganic nitrate in the consumed beetroot juice varied between 5.1 and 45 mmol/dose, corresponding to 316–2790 mg nitrate (142–1260% of the ADI value) [147]. In a randomized crossover trial, the effect of a 10-day period of consumption of Japanese traditional diet on blood pressure in 25 healthy volunteers was investigated. The authors reported that nitrate in the traditional Japanese diet (18.8 mg/kg b.w./day) lowered diastolic blood pressure in healthy volunteers on average by about 4.5 mmHg when compared to the control group, and this effect was suggested to explain in part the beneficial health effects of such foods [148]. Besides lowering the diastolic blood pressure at rest and during cardiopulmonary exercise, the consumption of 250–500 mL beetroot juice containing 5.1–11.2 mmol nitrate, i.e., 316–694 mg nitrate/person/day (142–310% of the ADI value) or 5.3–11.6 mg nitrate/kg b.w./day, was reported to increase exercise tolerance in healthy humans [149–152] and is supposed to be due to a reduced ATP turnover [153] or an increased mitochondrial efficiency [154]. Moreover, in humans with peripheral arterial disease

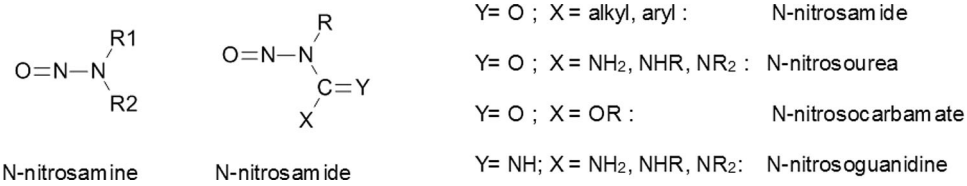

Figure 2. Structures of NOC.

characterized by intermittent disruption of blood and oxygen supply, 500 mL beetroot juice containing 9.1 mmol nitrate, i.e., 564 mg nitrate/person/day (254% of the ADI value) or 9.4 mg nitrate/kg b.w./day, significantly increased exercise performance, most probably due to an NO-mediated increase in tissue perfusion and oxygenation [155]. Thus, the reported beneficial health effects encompass high nitrate intakes exceeding the ADI value of 222 mg/person/day several-fold (i.e., in part by a factor greater than 10).

A reduction in blood pressure was noted in healthy volunteers after dietary supplementation with nitrate [146, 147], an effect consistent with the formation of vasodilating NO. In addition to vasodilation, mitochondrial function, leukocyte adhesion, and platelet aggregation also seem to be positively affected [109, 154].

Of note, randomized clinical intervention trials have indicated a reduction in blood pressure of about 5 mmHg to be significantly associated with decreased cardiovascular morbidity and mortality [156, 157], whereas a comparable increase was associated with the opposite effect [158].

4 Potentially detrimental health effects of NOC

In addition to their carcinogenic effects, NOC have been shown to exhibit mutagenic, teratogenic, and diabetogenic properties in several animal species [159–163]. In humans, the epidemiological evidence regarding the teratogenic effects of NOC is conflicting [164–172]. It was first shown in rats that oral administration of nitrosatable amino compounds together with nitrite can lead to the formation of malignant tumors indistinguishable from those induced by the corresponding *N*-nitroso carcinogen [173]. Furthermore, enhanced exposure to nitrate results in enhanced urinary excretion of *N*-nitrosated amino acids in humans [174, 175]. The latter are highly water-soluble noncarcinogenic NOC that are routinely used as (surrogate) biomarkers to monitor endogenous *N*-nitrosation under various conditions [174, 175]. However, despite many years of research, the potential health risk resulting from the endogenous formation of carcinogenic NOC has still not been assessed in detail up to the present time. This is in part due to the fact that validated biomarkers reflecting carcinogenic NOC formation are largely missing. DNA base adducts like O^6-carboxymethylguanine (O^6CMG) may be potentially useful biomarkers, reflecting endogenous formation of carboxymethylating/methylating agents from the interaction of primary amino groups in amino acids, peptides, or proteins with nitrosating agents (expanded in Section 4.1). Yet, the multitude of physiological and disease-related processes such as infections and/or inflammation, which may give rise to nitrosating agents, complicate any approach to study long-term health effects.

In addition, NOC may already be formed in foods, e.g., as a consequence of processing them with nitrite or nitrate (meat curing). Thus, humans are also exposed to exogenously formed NOC from various sources such as foods and cosmetics [176]. The WCRF/AICR reported "convincing" evidence that a high consumption of red and processed meat is associated with an enhanced risk of developing colorectal cancer, thereby suggesting, among other factors, a potential contribution of NOC to the formation of such tumors [177]. However, whether this association reflects enhanced exposure to NOC in meat or whether other factors such as nitrosylheme and nitrosothiols (see Section 4.4.3) may contribute, remains to be resolved.

4.1 Exogenous and endogenous formation of NOC

NOC comprise *N*-nitrosamines and *N*-nitrosamides (see Fig. 2). Whenever nitrosating agents encounter *N*-nitrosatable amino compounds, NOC may be formed. Nitrosation rate constants depend on the reaction mechanism involved, on the nature and concentration of the reaction partners, the reaction medium, pH value, and further modifying factors, including the presence of catalysts or inhibitors of *N*-nitrosation. The classical situation is reflected by the reaction of an amine with nitrous acid (HNO_2) in aqueous solution, as it prevails in the stomach during digestive gastric passage (pH 2.5–3.0) [19, 178, 179]. The HNO_2/nitrite equilibrium has a pK_a value of 3.3–3.4. Therefore, at a gastric pH of around 3 about half of the nitrite in the gastric fluid will be present in the protonated form as HNO_2. The latter equilibrates with the nitrosyl cation (NO^+) and nitrogen oxides, especially dinitrogen trioxide (N_2O_3), which represents the rate-limiting nitrosating species toward amines under these conditions (Fig. 3). Furthermore, N_2O_3 equilibrates with NO and NO_2, the latter in turn with dinitrogen tetroxide (N_2O_4), thereby causing nitrosation and nitration reactions. NO can combine with the superoxide anion radical to generate peroxynitrite ($ONOO^-$), a pivotal component of physiological reactive nitrogen species. The reactivity of peroxynitrous acid (ONOOH) is influenced by the bicarbonate/carbon dioxide equilibrium and by other physiological reactants, including low molecular weight thiols or heme centers. Under acidic

conditions, nitrosyl and nitrous acidium ions may be paired to a spectrum of anions, including halogenides, thiocyanate, and others. Depending on the pH, such counterions may act as catalysts of *N*-nitrosation or even act as nitrite scavengers, thus inhibiting NOC formation, depending on the specific scenario considered with respect to exogenous and in vivo formation of NOC. In the stomach, the nitrosating species for basic amines is N_2O_3, which arises under proton catalysis from two molecules of HNO_2. Therefore, the nitrosation rate depends on the square of the nitrite concentration, and the reaction follows second-order kinetics with respect to nitrite. As a consequence, enhanced nitrite concentrations will lead to sharply increased *N*-nitrosation rates under such gastric conditions. As can be deduced from Fig. 4, low pH favors formation of N_2O_3, but also increases amine protonation. Only nonprotonated nitrogen atoms are available for nitrosation. Thus, for most alkylamines the pH dependence of the *N*-nitrosation rate follows a bell-shaped curve, with peak rates around pH 3–4 [180]. Therefore, strongly basic mono-, di-, or trialkyl amines ($pK_a > 9.5$) are not considered to exhibit nitrosation rates that favor substantial NOC formation in acidic gastric media. Under such conditions, the concentration of the protonated species exceeds the concentration of the unprotonated species by factors of roughly 10^6 or more. Thus, weakly basic amines ($pK_a < 9.5$) are of great relevance for in vivo nitrosation in the acidic gastric medium [52, 60]. Specific considerations apply, when an aldehyde is present. For instance, under neutral to alkaline conditions formaldehyde has been shown to strongly catalyze the conversion of various secondary amines to nitrosamines, probably as a result of forming intermediate hydroxymethylamine adducts [181]. Because acid-catalyzed nitrosation in the absence of formaldehyde and, potentially, other foodborne aldehydes is inappreciable at pH > 5, NOC in food and certain consumer products or under specific working place conditions most likely arise from exposure of unprotonated amines to gaseous NO_x and/or other nitrosating agents potentially generated in food (e.g., *O* and *S*-nitroso compounds as well as nitrosyl heme intermediates, e.g., in cured meat products).

The *N*-nitrosation reaction can also be inhibited, e.g., in the presence of ascorbic acid, primary amines, tannins, or other phenolic compounds [182]. Thus, it has been discussed that, while some vegetables provide high intakes of nitrate, they also provide substantial intakes of food constituents that can at least partially inhibit the formation of NOC [183–185]. The protective potential of such dietary inhibitors depends not only on the reaction rates of *N*-nitrosatable precursors and nitrosation inhibitors, but also on their biokinetics, since a given inhibitor, in order to be effective, needs to follow gastrointestinal circulation kinetics similar to nitrate [186].

Nitrosamines most frequently found in food are *N*-nitrosodimethylamine (NDMA), *N*-nitrosopyrrolidine, *N*-nitrosopiperidine, and *N*-nitrosothiazolidine. Nonvolatile NOC mainly consist of *N*-nitrosated amino acids, including the *N*-nitroso products of sarcosine, NPRO, thiazolidine-4-carboxylic acid, oxazolidine-4-carboxylic acid, and *N*-nitroso-2-methyl-nitroso thiazolidine-4-carboxylic acid as well as the oxazolidine analog [187, 188]. The formation and occurrence of NOC in cosmetics and consumer products has recently been summarized in two opinions by the EU Scientific Committee on Consumer Safety (SCCS) [189, 190].

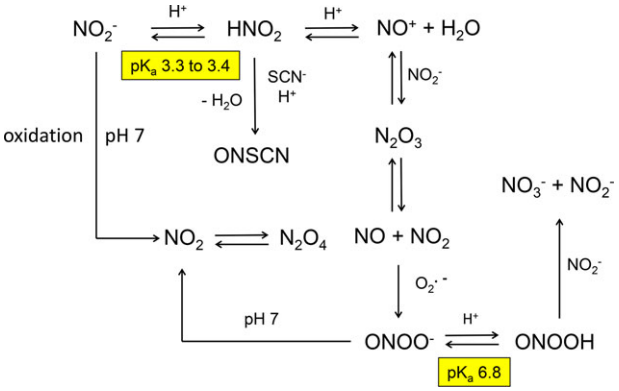

Figure 3. Main metabolic pathways of nitrite (modified according to [19]).

There is compelling evidence for endogenous formation of NOC in humans. Thus, formation of NOC is to be expected not only under environmental, technical, or household conditions, thereby favoring the reaction of nitrosatable amino compounds in food with nitrosating agents, but also after food ingestion, e.g., during the stomach passage due to the reaction of precursor amines or amides with nitrite. Similar reactions of secondary or tertiary amines or amides have been described after oral intake of drugs [191]. It has been shown that dietary nitrate and nitrite can lead to NO formation in the stomach and the large intestine [192–194]. NO is not a *N*-nitrosating agent per se, but may be rapidly converted into nitrosating and oxidizing reactants such as NOx and peroxynitrite. In addition, certain infections, which lead to inflammatory processes, may also contribute to endogenous nitrosation reactions [36], as shown, e.g., by an increased urinary elimination of nitrosated amino acids. An early study by Sander and Bürkle in 1969 proved that endogenous NOC formation can occur in vivo. When weakly basic amines like *N*-methylbenzylamine and morpholine were given together with nitrite to rats, the same tumors as observed after application of the corresponding NOC were induced [173]. Further indications came from the observation that malignant liver and lung tumors developed in rats fed the drug amidopyrine (AP) together with nitrite. This was attributed to the extremely high reactivity of AP toward nitrosating agents, releasing NDMA by endogenous nitrosation [195]. The in vivo nitrosation of AP in humans was proven after ingestion of AP together with a nitrate-rich vegetable (radish). The concomitant intake of ethanol (in 500 mL beer) prevented CYP 450-mediated NDMA metabolic clearance, which occurred very rapidly in the absence of ethanol [196, 197]. Under these experimental conditions, NDMA formed from AP was protected

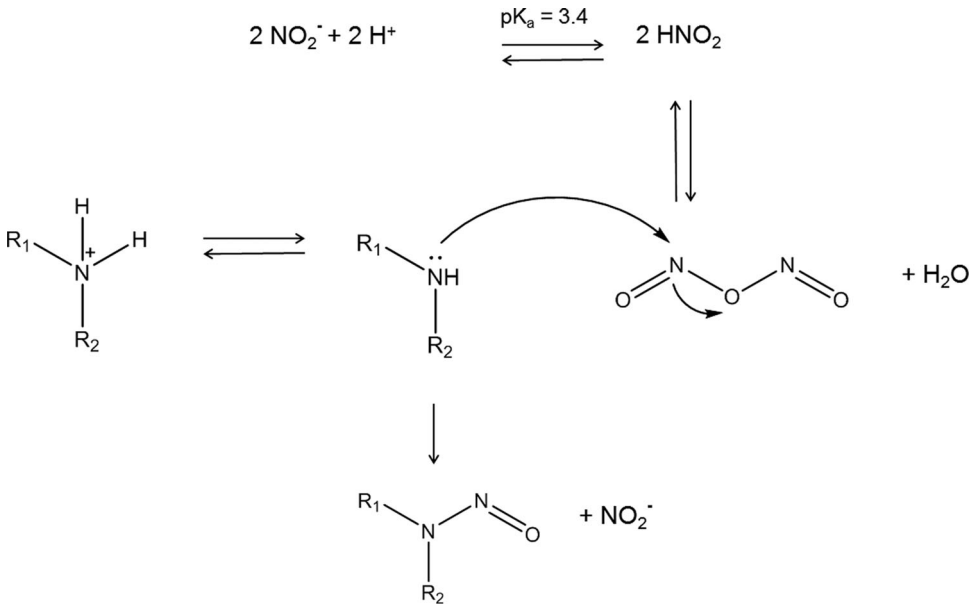

Figure 4. Mechanism of nitrosation of secondary amines [60].

from CYP 450-dependent metabolic clearance and therefore became detectable in the urine of the volunteers [196, 197]. This confirms the premise that nitrate taken up with the diet undergoes enterosalivary circulation and partial conversion to nitrite in the oral cavity, leading to the endogenous nitrosation of *N*-nitrosatable precursors from food (Fig. 5). NDMA formation from AP was further confirmed by an ex vivo study, separately collecting human saliva samples from differently exposed volunteers. From one group of volunteers, saliva was obtained after ingestion of a nitrate-rich vegetable juice containing 200 mg nitrate. From the second group of volunteers, saliva was collected for 0.5–5 h after intake of AP at the recommended daily dose of 500 mg. Saliva samples from both groups were mixed and incubated under simulated gastric conditions (15 min, pH 3, 37°C). NDMA formation in the combined saliva mixture was detected as early as 30 min after AP intake, with a maximum yield (980 ng NDMA/mL) 2.5 h after ingestion, still measurable 5 h after ingestion (300 ng NDMA/mL) [197]. This clearly demonstrates that formation of NOC in the gastrointestinal tract is to be expected when a high dietary nitrate intake coincides with the ingestion of easily nitrosatable precursors. Enterosalivary recycling of both components and partial reduction of nitrate to nitrite by the oral microbiota, as exemplified in the case of AP, plays a major role (Fig. 5).

Biomarkers to determine the nitrosation in the gastrointestinal tract need to be developed. The nitrosation of proline to NPRO has been used, since endogenously formed NPRO is quantitatively excreted in urine. However, under certain circumstances, e.g., when gastric acid levels are low in the stomach (pH > 4), nitrosation of proline does not occur [198], whereas NDMA would still be generated from AP.

The exposure of laboratory animals to tobacco constituents may serve as a model to study different aspects related to the carcinogenicity of nitrosamines to humans. The concomitant exposure of rats to nornicotine and nitrite was shown to result in the endogenous formation of *N*-nitrosonornicotine (NNN), a tobacco-specific nitrosamine [199]. NNN is easily and rapidly formed by nitrosation of nornicotine and less readily by nitrosation of the tertiary amine nicotine [200, 201].

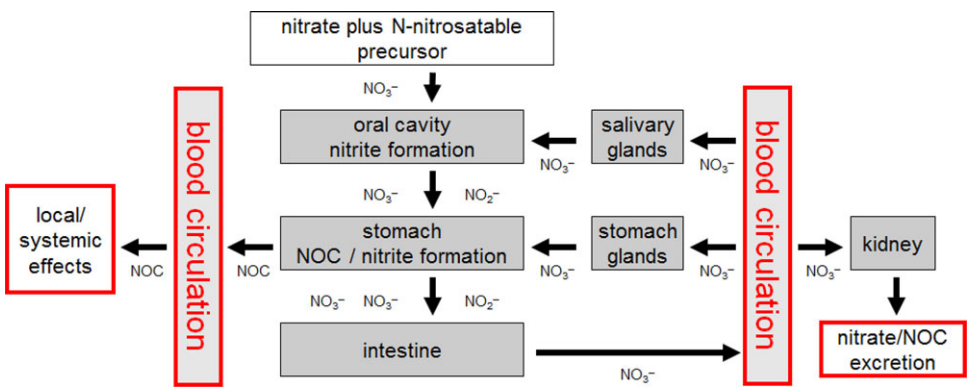

Figure 5. Nitrate ingestion, conversion to nitrite, and gastric NOC formation.

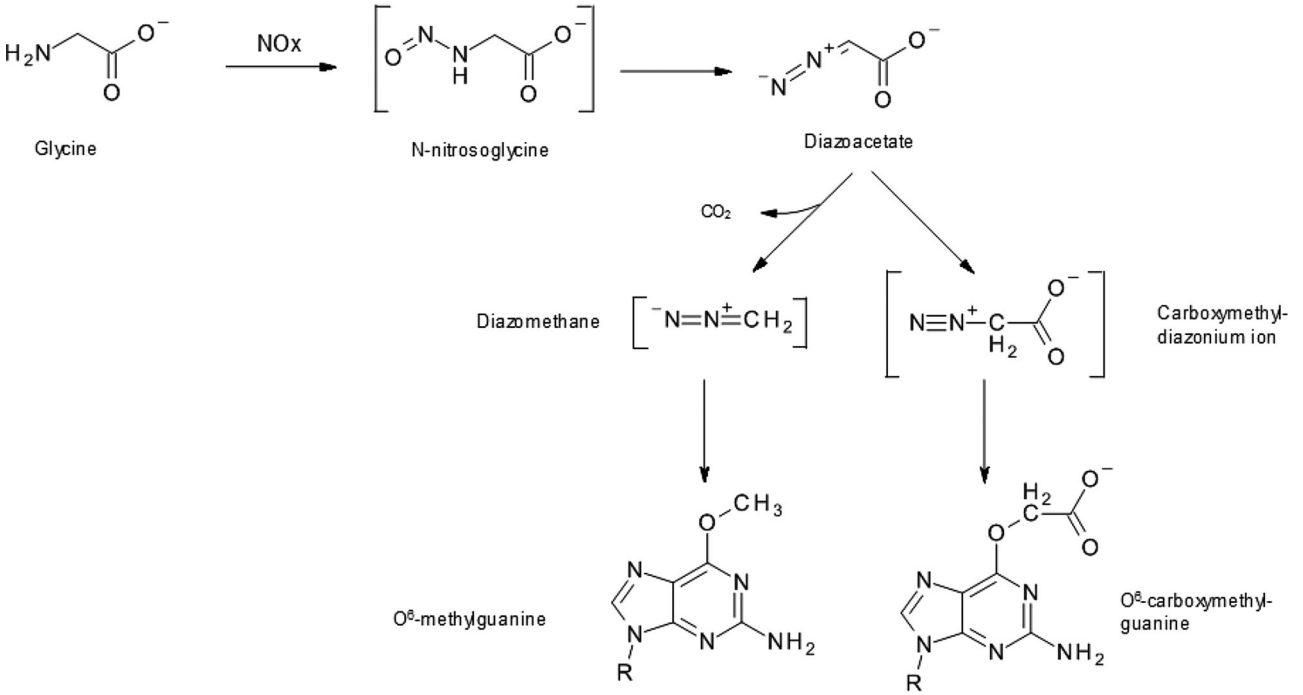

Figure 6. Nitrosation of glycine and formation of guanine adducts (modified according to [212]).

NNN, a highly potent tobacco carcinogen, is not found in the diet or in the general environment, except when tobacco smoke is present. Monitoring urinary mercapturic acids as biomarkers provided evidence for the endogenous formation of NNN in humans taking nicotine replacement products [202, 203]. This potential hazard could be addressed, at least in part, by excluding nornicotine contamination from nicotine replacement products or by combining them with nitrosation inhibitors. There is presently no evidence that long-term users of nicotine replacement products are at increased risk of cancer above and beyond that due to their history of smoking. Nevertheless, it would be prudent to avoid exposing the users of these products to NNN.

Evidence from prospective human cohort studies revealed a remarkably strong association between urinary total NNN as biomarker of human NNN intake and the risk of developing esophageal cancer among smokers [204, 205]. This is consistent with evidence from animal carcinogenicity studies, showing potent carcinogenic effects of NNN and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in rat esophagus and lung [206]. NNN and NNK have been classified as "human carcinogens" (group 1) by the International Agency for Research on Cancer (IARC) [207].

Carboxymethylating/methylating agents associated with dietary nitrosating agents may play a potential role in gastrointestinal carcinogenesis. *N*-nitroso glycine derivatives have been found to be mutagenic in bacterial test systems and human lymphoblasts [208]. As an example, *N*-nitrosoglycocholic acid was found to induce gastric cancer in experimental animals [209]. The formation of certain adducts in human DNA, e.g., O^6-carboxymethyl-2′-deoxyguanosine (O^6CMdG) and O^6-methyl-2′-deoxyguanosine (O^6MedG), has been assumed to be a consequence of the nitrosation of glycine (or glycine-containing substrates) (Fig. 6) [210]. *N*-nitrosation of glycine results in the formation of diazoacetate or its analogues, giving rise to the formation of O^6CMG. It has been reported that O^6CMG adducts are not repaired by O^6-alkylguanine-DNA-alkyltransferases and may therefore accumulate in the DNA of gastrointestinal tract tissues [210], while a recent report showed that O^6CMG can be repaired by the above-mentioned enzymes [211]. The in vitro pattern of mutations in the tumor suppressor gene *p53* exposed to potassium diazoacetate has been found to be similar to the pattern of *p53* mutations observed in human gastrointestinal tumors [212]. Antibody-based assays have been developed for the detection of O^6CMdG, including immunoaffinity-HPLC, immuno-slot blot assays, and immunohistochemistry [213–215]. More recently, sensitive MS-based assays have become available [216]. Future prospective studies on colorectal cancer risk may use O^6CMdG as a potential biomarker. Primary amino acids other than α-amino acids, such as β-amino (e.g., β-alanine) or γ-amino (e.g., γ-amino butyric) acids may give rise to the corresponding lactones under nitrosating conditions. Knowledge of the reactivity of those amino acids may be useful to predict potentially adverse effects of such non-NOC nitrosation products.

The endogenous nitrosation of 5,6-dihydrouracil (DHU), a physiological metabolite of pyrimidine bases (DNA, RNA) present in human urine and plasma, yields 1-nitroso-DHU [179], which is a powerful rat hepatocarcinogen [217].

Furthermore, it has been suggested that this NOC is involved in the formation of the DNA adduct 7-carboxyethylguanine (7-CEG) [218]. Rats fed DHU or β-ureidopropionic acid together with nitrite in drinking water showed a significant increase of 7-CEG levels in hepatic DNA. This is indicative of an endogenous nitrosation, resulting in the formation of direct DNA damaging agents. *N*-methylnitrosamino propionic acid as well as acrylic acid, presumed to be a metabolite of acrolein, have also been proposed to contribute to the formation of 7-CEG adducts in human liver DNA. The latter is a common environmental contaminant and also is an endogenous product of lipid peroxidation [218]. Thus, the endogenous nitrosation of DHU and methylamino propionic acid to the corresponding NOC as well as the endogenous and/or exogenous exposure to acrolein may therefore serve as sources for 7-CEG adducts found in human tissues.

The intragastric formation of NDMA after dietary nitrate intake was also investigated in the in vitro dynamic digestion model TIM1 simulating the upper gastrointestinal tract (TNO Nutrition and Food Research, Zeist, The Netherlands). It consists of a computer controlled in vitro flow-through system that mimics the physiological processes in the human stomach and small intestine during digestion. After adding fish as a model food rich in amines together with nitrite to the stomach compartment, formation of NDMA was monitored. Under these in vitro conditions, a minimal amount of NDMA was formed. It was assessed to result in a margin of exposure (MOE, see Section 5.3) higher than 100 000 [219, 220]. These results suggest that the assessment of nitrosamine formation in such gastrointestinal tract models using food rich in strongly basic amines most likely underestimates the risk associated with endogenous NOC formation, in line with the premise that strongly basic secondary or tertiary amines in food like di- or trimethylamine in fish are not efficiently nitrosated. The mean daily dietary intake of total primary and secondary amines has been reported to be 30 and 7 mg/day, respectively [221]. In addition, there is evidence for the contribution of gut microbiota to endogenous NOC formation [47, 48, 222].

4.2 Structure/activity relationship of NOC

NOC are a class of potent human carcinogens. The carcinogenic potential of NDMA was already described in 1956 [223], that of the homologue *N*-nitrosodiethylamine (NDEA) only a few years later [224]. In 1967, Druckrey and colleagues published a comprehensive study on the carcinogenicity as well as the structure–activity and dose–response relationship of 65 NOC in BD rats [225]. The outstanding carcinogenic potency of NDMA and NDEA has been compellingly established in comprehensive, lifelong animal studies [226–229]. Since bioactivation and interaction with critical cellular targets is similar in animals and humans, NOC are regarded as presumed human carcinogens. The carcinogenic potential of NOC depends on their structure. NDMA, NDEA, and the tobacco-specific NNK are among the most potent carcinogens. This is reflected by BMDL10 (where BMDL10 is the bench mark dose lower confidence limit 10%) values of 18 and 27 μg/kg b.w. for NDEA and NDMA, respectively [189] (see also Section 4.3). Regarding tumorigenicity, NNK is considered to be about equally as potent as NDMA and NDEA [207, 230]. In terms of extrapolation from animal experiments to humans, it needs to be taken into consideration that organ-specific effects of NOC are species-dependent. Therefore, a reliable extrapolation of data regarding organ-specific effects from one species to another, including humans, is not possible without further detailed molecular, mechanistic, and biokinetic information [231].

Preformed and endogenously formed *N*-nitrosamines are well absorbed in the gastrointestinal tract [232–236]. The first step in the metabolic activation of NOC is the hydroxylation at the α-C position, mediated by several CYP 450 monooxygenases. The resulting α-hydroxy-*N*-nitrosamine, a proximal carcinogen, is unstable and rapidly dissociates into an aldehyde and a monoalkylnitrosamine, the latter rearranging into the corresponding diazonium intermediate. The diazonium electrophile can react with cellular macromolecules such as DNA, RNA, or protein, forming covalent adducts with appropriate nucleophilic centers (Fig. 7). Metabolically generated reactive electrophilic compounds lead to the alkylation of DNA bases, mainly at the N^7, O^6, and N^3 positions of guanine, at the N^1, N^3, and N^7 positions of adenine, and at the O^2 and O^4 positions of thymine. The DNA damaging effect is generally accepted to represent the key event initiating a chain of biological responses finally leading to cancer. Among the different DNA base adducts, N^7-alkylguanine, in general, is predominant. However, O^6-alkylguanine as well as O^4- and O^2-alkylpyrimidine adducts, which cause DNA mismatches and miscoding, are more potent mutagenic lesions potentially leading to carcinogenesis.

The basic structural requirements for the carcinogenicity of NOC have extensively been reviewed [231, 237]. Since the α-C position is crucial for metabolic activation, branching in this position reduces carcinogenicity. A tertiary substituent in this position completely inhibits carcinogenicity [231]. In addition, the chain length and symmetry of NOC has a great influence on their organotropy. Symmetric NOC mainly induce tumors in the liver, whereas asymmetrically substituted NOC lead to carcinomas in the esophagus and/or the urinary bladder (longer side chains) [231, 237].

4.3 Risk assessment of nitrosamines, MOE

Common NOC have been ranked with respect to their carcinogenic potential by using dose descriptors (BMDL10, T25) prevailing in carcinogenic risk assessment [188, 238]. The SCCS of the European Commission has used high-quality data [226, 227, 229] in its dose–response modeling [189]. The benchmark dose leading to a 10% tumor incidence in rats (BMD10 or BMDL10, taking into account the 95% lower

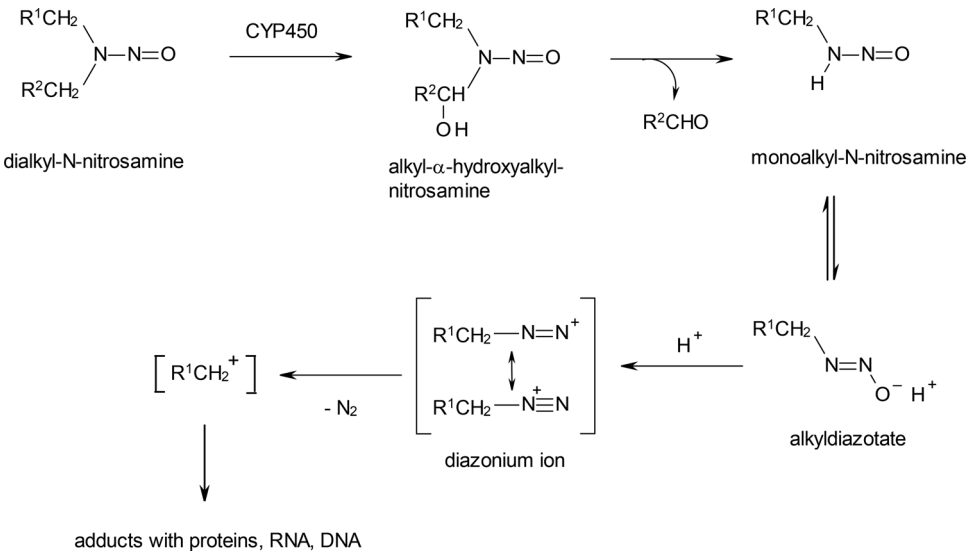

Figure 7. Metabolic activation of dialkyl-*N*-nitrosamine to an electrophilic alkylating agent [[187]; with permission from Wiley & Sons].

confidence limit) revealed NDEA and NDMA to represent by far the most potent compounds, exhibiting BMDL10 values of 18 and 27 μg/kg b.w./day, respectively [189]. For comparison, BMDL10 values of other potent foodborne carcinogens have been reported to range between 120 (benzo[*a*]pyrene) and 480 μg/kg b.w./day (2-amino-1-methyl-6-phenylimidazo [4,5-*b*]pyridine) [239]. These may be used to calculate a MOE, which describes the ratio between human exposure and a dose level inducing a defined tumor response in animals (in this case a 10% response). According to EFSA, a MOE of 10 000 or higher would be of low concern for health risk management [240]. Data on the occurrence of NOC in processed foods are rather outdated, being mostly from the 1980s to the 1990s. In these years, a significant reduction of NOC levels in foods and cosmetics was achieved due to appropriate mitigation measures. Human dietary intake of volatile NOC in Germany has been estimated to be 0.2–0.3 μg/person/day (3.3–5.0 ng/kg b.w./day based on a b.w. of 60 kg) [176]. The MOE resulting from NDMA exposure can be assessed to be in the range from 5400 to 8200. Taking into account an additional exposure of 0.4 ng/kg b.w./day NDMA formed endogenously from food components, as estimated from an in vitro gastrointestinal tract model [220], the MOE would marginally decrease to 5000–7300. However, as mentioned in the last paragraph of Section 4.1, the formation of NDMA from strongly basic amines such as dimethylamine in in vitro gastrointestinal tract models most likely underestimates the endogenous NOC formation, so that the MOE value could in fact be lower than the above-mentioned one. To which extent the MOE value could further be lowered cannot be deduced from the presently available data. Clearly, there is also a need to update the database on NOC levels in food in order to support assessment of overall exposure and risk. However, dimethylamine or diethylamine as potential precursors of NDMA or NDEA are not considered relevant indicators of endogenous NOC formation from foods. Therefore, adequate biomarkers still need to be identified.

4.4 Nitrate, nitrite, NOC, and cancer

4.4.1 Animal studies

The carcinogenicity of sodium nitrate was investigated in a 2-year study [241]. Groups of 50 male and 50 female Fischer 344 rats, 8 weeks of age, were fed a diet containing 0, 2.5, or 5% sodium nitrate for 2 years, equivalent to 0, 1250, or 2500 mg sodium nitrate/kg b.w./day or 0, 910, or 1820 mg nitrate ion/kg b.w./day. The incidence of mononuclear cell leukemia was reduced in the treated groups when compared to controls (males: control group, 36%; low-dose group, 4%; high-dose group, 2%; females: control group, 28%; low-dose group, 0%; high-dose group, 2%). No significant differences were observed in the incidence of any other types of tumors. IARC reviewed the carcinogenicity data on nitrate and noted that Fischer 344 rats show a high incidence of spontaneous mononuclear cell leukemia. It was concluded that there is inadequate evidence in experimental animals for the carcinogenicity of nitrate [242].

The carcinogenicity of sodium nitrite was investigated by the U.S. National Toxicology Program in a 2-year rat and mouse study [243]. Groups of 50 male and 50 female rats were exposed to 0, 750, 1500, or 3000 ppm sodium nitrite (equivalent to average daily doses of approximately 0, 35, 70, or 130 mg sodium nitrite/kg b.w. in the case of males and 40, 80, or 150 mg sodium nitrite/kg b.w. in the case of females) in drinking water for 2 years. The U.S. National Toxicology Program study concluded that there was no evidence of carcinogenic activity for sodium nitrite in male or female F344/N rats and in male B6C3F_1 mice [243]. Equivocal evidence of carcinogenic activity was reported for sodium nitrite in female B6C3F_1 mice, based on the positive trend in the incidences of squamous cell papilloma or carcinoma (combined) in the forestomach [243]. IARC reviewed the carcinogenicity of nitrite and concluded that there is limited evidence in experimental animals for the carcinogenicity of nitrite per

se [242]. However, IARC also stated that there is sufficient evidence in experimental animals for the carcinogenicity of nitrite in combination with amines or amides [242].

4.4.2 Evidence in humans

The relevance of the endogenous formation of NOC for human carcinogenesis remains a matter of debate. Experimental models demonstrating that NOC can induce tumors in the gastrointestinal tract are available, but there is little evidence that exposure to such compounds is directly involved in the induction of such tumors in humans. Indeed, many NOC do not directly lead to malignant cell transformation. Instead, they need to be absorbed to become metabolically activated, thereby inducing cancer at sites distant from the site of formation or incorporation. Still, in vitro experiments have shown that mutations induced by compounds generated by endogenous nitrosation are similar to those found in colorectal tumors, supporting the hypothesis that endogenous nitrosation may be one causative mechanism [212]. IARC concluded that ingested nitrate or nitrite under conditions that result in endogenous nitrosation is probably carcinogenic to humans (Group 2A) [242].

More recent evidence reported a significant association between urinary NOC excretion and micronuclei frequency in human lymphocytes as well as gene expression changes associated with malignant cell transformation in NOC-exposed Caco-2 cells [244, 245]. Whole blood transcriptomes of 30 individuals were screened for transcription patterns correlating with NOC exposure markers and micronuclei frequency in lymphocytes [245]. In a network analysis, various genes were identified as potential transcriptomic biomarkers. The association between NOC excretion and micronuclei frequency suggested that an increased cancer risk for humans exposed to NOC may exist. If confirmed, the identified genes may be used in future studies as genetic markers for NOC exposure. Gene expression analysis in colon biopsies of inflammatory bowel disease and irritable bowel syndrome patients demonstrated a correlation between fecal NOC levels and gene expression changes [246]. Genes associated with chromatin assembly were found to be negatively or positively correlated with the level of apparent total nitroso compounds (ATNCs). The determination of ATNC is based on the release of NO from a given medium. Depending on the chemistry of the pretreatment prior to NO release, it may also detect compounds other than NOC that may contribute to NO formation [247]. Intake of red meat was found to be associated with increased fecal water genotoxicity and with particular gene expression changes. These include the gene coding for protein kinase A as well as genes associated with cytoskeleton reorganization, the WNT signaling pathway and small ubiquitin-related modifiers (SUMO), significant regulators of the response to DNA damage [248]. Taken together, the exposure of Caco-2 cells to NOC led to changes in gene expression associated with malignant cell transformation, while the incubation of human blood cells with NOC induced the formation of micronuclei, a biomarker of DNA damage.

4.4.3 Red/processed meat

The consumption of red and processed meat may result in an intake-dependent endogenous formation of ATNC [249, 250]. The main contributors to ATNC formation following the consumption of a high meat diet appear to be nitrosyl heme and nitroso thiols [251]. Major sources for dietary heme are roast beef, steaks, hamburgers, sausages, and ham. The amount of ATNC in feces was found to correlate with red meat intake, and additional heme intake further increased the ATNC level [250]. While it is known that free thiol and heme groups are necessary for NOC to be formed, the mechanisms underlying the meat-induced endogenous formation of NOC have not yet been completely elucidated. Both nitrosyl heme and nitroso thiols are known to act as NO donors and both can act as nitrosating agents [252, 253]. Therefore, these compounds probably stimulate the formation of NOC or highly reactive alkylating intermediates, which eventually may lead to DNA damage.

In some human dietary intervention studies, a strong correlation between endogenous NOC formation and the presence of O^6CMG adducts has been reported [215]. In fact, O^6CMG adducts were detected in exfoliated colonic cells of volunteers consuming high amounts of red meat, and the adduct levels were significantly higher than those of volunteers on a vegetarian diet. In this context, it should be noted that *N*-nitrosation of glycine leads to the formation of diazoacetate or its analogues, which may give rise to the formation of O^6CMG. A study in 185 archived colorectal cancer tumor samples (EPIC Norfolk) reported a strong association between meat intake and GC-to-AT transition mutations [254]. These data support the hypothesis that endogenous nitrosation reactions leading to the formation of alkylating agents and DNA damage are likely to contribute to the association found between the consumption of red and processed meat and colon cancer risk. The potential role of red/processed meat in colorectal cancer development has recently been reviewed [255].

Prospective cohort studies suggest an association of red meat and nitrite-preserved meat intake with an increased risk for colon cancer [251, 256–259]. One of the studies used the outcome of a continuing survey of food intake in the United States to create meat categories for smoked and processed meat. The content of nitrate and nitrite was estimated by using chemical analysis, standard recipes for meat processing, and literature values. The data of a food frequency questionnaire regarding intakes of processed meat and residual nitrate and nitrite were used to estimate the nitrate and nitrite intake [260–262]. In a large prospective cohort study (NIH-AARP) the role of red and processed meat, nitrate, and nitrite in cancer development was assessed. Elevated risks were reported for red and processed meat (nitrite) intake in

association with colorectal cancer [263], and for nitrate/nitrite intake in association with several types of human cancer including tumors of the thyroid gland, ovary, kidney, and bladder [262, 264–267]. No associations were reported for other cancer types [257, 264–266, 268, 269]. Findings were controversial for glioma [270–272]. In the EPIC study, endogenous NOC (calculated from iron intake with meat and fecal ATNC formation) and dietary nitrite were not significantly associated with cancer risk [273, 274].

In conclusion, the epidemiological evidence regarding the role of endogenous NOC formation for human cancer risk is inconsistent. Obviously, there is a need for more elaborate studies, which make use of appropriate biomarkers and also include those determined by applying "omics" technologies, in order to establish causality for the association.

Future comprehensive studies need to take into consideration, among other parameters, nitrate/nitrite exposure and the extent of NOC formation in vivo, the nature and relevance of *N*-nitrosatable precursors and the resulting NOC, the influence of individual dietary and physiologic factors, but also the individual health status, especially with respect to those conditions favoring endogenous NOC formation including inflammatory and/or infectious diseases.

Such comprehensive studies, preferentially in humans, may be integrated into well-designed prospective (molecular) epidemiologic studies or may be carried out as double-blind, randomized controlled intervention studies. Long-term as well as short-term health effects need to be considered.

5 Conclusions, recommendations, and research needs with respect to risk/benefit assessment

The SKLM is of the opinion that there is a need for further research addressing potentially negative and positive health effects associated with dietary nitrate and nitrite exposure. The available evidence is inadequate to be used as a basis for a comprehensive and reliable assessment of positive as well as negative health effects, especially regarding long-term effects. Human intervention studies should be undertaken, making use of appropriate biomarkers for potentially detrimental or beneficial health effects. Such studies also need to consider individual differences such as age and gender, genetic background, and health status as well as the role of infections and inflammatory processes.

5.1 Biomarkers reflecting nitrate/nitrite associated beneficial/adverse effects

To allow an adequate evaluation of the consequences of a dietary exposure to nitrate/nitrite for human health, an array of predictive and reliable biomarkers should be developed. Regarding beneficial effects related to the intake of nitrate/nitrite, monitoring of mean diastolic blood pressure is proposed as a surrogate short-term biomarker predictive for long-term beneficial effects, especially reduced mortality due to CVDs. In addition to blood pressure, further biomarkers related to CVD risk, such as those reflecting effects on circulation/blood flow, platelet adhesion/aggregation, and maintenance of vessel tonus, should also be taken into consideration.

At the same time, there is an urgent need to adequately characterize the potential health risk associated with an enhanced dietary nitrate intake and to develop biomarkers indicative of endogenous as well as exogenous NOC exposure. As an easily accessible surrogate biomarker for overall endogenous *N*-nitrosation, the urinary excretion of noncarcinogenic *N*-nitroso amino acids may be utilized. However, it needs to be established to what extent monitoring of urinary noncarcinogenic *N*-nitroso amino acids can also be taken as a biomarker for the endogenous formation of carcinogenic NOC or related genotoxic agents. Potentially negative effects of NOC are mainly considered to result from genotoxic DNA damage resulting in mutations and finally in cancer. To corroborate the correlation of such biomarkers with the risk resulting from the in vivo generation of carcinogenic NOC or corresponding genotoxic intermediates, specific DNA adducts as indicators of genotoxic damage by NOC in human blood leucocytes or biopsy samples need to be monitored. Moreover, specific transcriptomic responses as indicators of genotoxic damage and subsequent biological responses, including DNA repair and mutation induction, should be investigated in human blood and/or tissue samples and assessed for their value as predictive biomarkers.

The SKLM realizes that the predictive power of these biomarkers will have to be validated in the future by appropriate prospective molecular epidemiology studies.

5.2 Human intervention studies

The SKLM is of the opinion that well-designed dietary intervention studies constitute an essential step in the process of accomplishing a reliable risk/benefit assessment with respect to the long-term human health effects of nitrate/nitrite. Such human intervention studies should be performed in subpopulations at an enhanced health risk, e.g., in slightly hypertensive individuals. It is known that a reduction of 5 mmHg is sufficient to significantly reduce the long-term risk of developing CVDs. An intervention study in which volunteers ingest nitrate/nitrite at a level appropriate to achieve a reliably measurable and significantly beneficial reduction of the mean diastolic blood pressure may be envisaged.

5.3 Specific research needs

The outcome of human studies should constitute an essential part of a comprehensive database to be established on nitrate/nitrite-related beneficial as well as detrimental health effects and their dose dependency. In order to achieve this

objective, particular attention should be given to the following issues:

(i) to deduce potentially minimal effective doses of dietary nitrate that can significantly reduce blood pressure, e.g., from intervention studies in slightly hypertensive subpopulations;
(ii) to develop biomarkers for further potentially beneficial long-term effects;
(iii) to identify specific transcriptomic responses as an indication of short/long-term human health effects;
(iv) to establish biomarkers that reflect the endogenous formation of carcinogenic NOC;
(v) to explore the influence of the health status, especially bacterial infections and inflammatory diseases, on biomarker response;
(vi) to update the database on human dietary intake of nitrate/nitrite and especially NOC;
(vii) to explore the (endogenous) nitrosation kinetics of an array of amino compounds, which reflect the range of chemicals humans are exposed to and can plausibly be expected to act as precursors for carcinogenic NOC and/or to give rise to toxicologically relevant amounts of genotoxic electrophils in vivo;
(viii) to more firmly establish the relationship between overall NOC exposure, both from endogenous and exogenous sources, and the induction of cancer;
(ix) to establish methods to quantify risks as well as benefits to enable a reliable risk/benefit assessment.

The Senate Commission gratefully acknowledges the contribution of the following experts invited to the round table meeting "Nitrate and Nitrite in the Diet, Benefit/Risk for Human Health" held in Bonn, Germany, on November 27, 2012: Amrita Ahluwalia (Queen Mary University, London, UK), Stephen Hecht (University of Minnesota, Minneapolis, USA), Theo de Kok (University of Maastricht, Maastricht, The Netherlands), Gunter Kuhnle (University of Reading, Reading, UK), Jon Lundberg (Karolinska Institute, Stockholm, SE), David Shuker (The Open University, Milton Keynes, UK), Rashmi Sinha (National Cancer Institute, NIH, Rockville, USA), Marco Zeilmaker (RIVM, Bilthoven, The Netherlands).

Members and guests of the DFG Senate Commission on Food Safety 2011–2013 are as follows: *Gerhard Eisenbrand (Chairman), Patrick Diel, Karl-Heinz Engel, Johanna Fink-Gremmels, Jan G. Hengstler, Christian Hertel, Hans-Ulrich Humpf, Hans-Georg Joost, Dietrich Knorr, Sabine Kulling, Alfonso Lampen, Doris Marko, Gerhard Rechkemmer, Ivonne Rietjens, Richard H. Stadler, Pablo Steinberg, Stefan Vieths.*

Members and guests of the DFG Senate Commission on Food Safety 2014–2016 are as follows: *Pablo Steinberg (Chairman), Patrick Diel, Gerhard Eisenbrand, Karl-Heinz Engel, Bernd Epe, Peter Fürst, Volker Heinz, Hans-Ulrich Humpf, Hans-Georg Joost, Dietrich Knorr, Theo de Kok, Sabine Kulling, Alfonso Lampen, Doris Marko, Gerhard Rechkemmer, Ivonne Rietjens, Richard H. Stadler, Stefan Vieths, Rudi Vogel.*

The authors have declared no conflict of interest.

6 References

[1] SKLM, in: Forschungsgemeinschaft, D. (Ed.), *Lebensmittel und Gesundheit: Sammlung der Beschlüsse, Stellungnahmen und Verlautbarungen aus den Jahren 1984 bis 1996, Senatskommission zur Bewertung der gesundheitlichen Unbedenklichkeit von Lebensmitteln (SKLM)*, Wiley VCH, Weinheim 1998, pp. 77–86.

[2] World Cancer Research Fund/American Institute for Cancer Research, *Expert Report. Food, Nutrition, Physical Activity and the Prevention of Cancer: A Global Perspective*. AICR, Washington, DC 2007. Available from: http://www.aicr.org/assets/docs/pdf/reports/Second_Expert_Report.pdf.

[3] Weitzberg, E., Lundberg, J. O., Novel aspects of dietary nitrate and human health. *Annu. Rev. Nutr.* 2013, *33*, 129–159.

[4] Gilchrist, M., Shore, A. C., Benjamin, N., Inorganic nitrate and nitrite and control of blood pressure. *Cardiovasc. Res.* 2011, *89*, 492–498.

[5] Hord, N. G., Tang, Y. P., Bryan, N. S., Food sources of nitrates and nitrites: the physiologic context for potential health benefits. *Am. J. Clin. Nutr.* 2009, *90*, 1–10.

[6] Machha, A., Schechter, A. N., Dietary nitrite and nitrate: a review of potential mechanisms of cardiovascular benefits. *Eur. J. Nutr.* 2011, *50*, 293–303.

[7] Lidder, S., Webb, A. J., Vascular effects of dietary nitrate (as found in green leafy vegetables and beetroot) via the nitrate-nitrite-nitric oxide pathway. *Brit. J. Clin. Pharmacol.* 2013, *75*, 677–696.

[8] Greer, F. R., Shannon, M., Infant methemoglobinemia: the role of dietary nitrate in food and water. *Pediatrics* 2005, *116*, 784–786.

[9] European Food Safety Authority (EFSA), Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the European Commission to perform a scientific risk assessment on nitrate in vegetables. *EFSA J.* 2008, *689*, 1–79.

[10] Scientific Committee for Food (SCF), Opinion on nitrate and nitrite. *Reports of the Scientific Committee for Food (SCF), Series 26*, European Commission, Brussels 1992, pp. 21–28.

[11] Scientific Committee for Food (SCF), Opinion on nitrate and nitrite. *Reports of the Scientific Committee for Food (SCF), Series 38*, European Commission, Brussels 1997.

[12] FAO/WHO, *Nitrate (and Potential Endogenous Formation of N-Nitroso Compounds). WHO Food Additive Series No. 50*, World Health Organisation, Geneva 2003.

[13] European Food Safety Authority (EFSA), Statement on nitrites in meat products. *EFSA J.* 2010, *8*, 1538.

[14] FAO/WHO, *Nitrite (and Potential Endogenous Formation of N-Nitroso Compounds). WHO Food Additive Series No. 50*, World Health Organisation, Geneva 2003.

[15] Caulfield, J. L., Wishnok, J. S., Tannenbaum, S. R., Nitric oxide-induced deamination of cytosine and guanine in deoxynucleosides and oligonucleotides. *J. Biol. Chem.* 1998, *273*, 12689–12695.

[16] Burney, S., Caulfield, J. L., Niles, J. C., Wishnok, J. S. et al., The chemistry of DNA damage from nitric oxide and peroxynitrite. *Mutat. Res.* 1999, *424*, 37–49.

[17] Eiserich, J. P., Patel, R. P., O'Donnell, V. B., Pathophysiology of nitric oxide and related species: free radical reactions and modification of biomolecules. *Mol. Aspects Med.* 1998, *19*, 221–357.

[18] Gal, A., Tamir, S., Kennedy, L. J., Tannenbaum, S. R., Wogan, G. N., Nitrotyrosine formation, apoptosis, and oxidative damage: relationships to nitric oxide production in SJL mice bearing the RcsX tumor. *Cancer Res.* 1997, *57*, 1823–1828.

[19] d'Ischia, M., Napolitano, A., Manini, P., Panzella, L., Secondary targets of nitrite-derived reactive nitrogen species: nitrosation/nitration pathways, antioxidant defense mechanisms and toxicological implications. *Chem. Res. Toxicol.* 2011, *24*, 2071–2092.

[20] Knutson, C. G., Mangerich, A., Zeng, Y., Raczynski, A. R. et al., Chemical and cytokine features of innate immunity characterize serum and tissue profiles in inflammatory bowel disease. *Proc. Natl. Acad. Sci. USA* 2013, *110*, 2332–2341.

[21] Green, L. C., Deluzuriaga, K. R., Wagner, D. A., Rand, W. et al., Nitrate biosynthesis in man. *Proc. Natl. Acad. Sci. USA* 1981, *78*, 7764–7768.

[22] Tannenbaum, S. R., Fett, D., Young, V. R., Land, P. D. et al., Nitrite and nitrate are formed by endogenous synthesis in human intestine. *Science* 1978, *200*, 1487–1489.

[23] Weitzberg, E., Lundberg, J. O., Nonenzymatic nitric oxide production in humans. *Nitric Oxide* 1998, *2*, 1–7.

[24] Reutov, V. P., Sorokina, E. G., NO-synthase and nitrite-reductase components of nitric oxide cycle. *Biochemistry (Mosc.)* 1998, *63*, 874–884.

[25] Lundberg, J. O., Govoni, M., Inorganic nitrate is a possible source for systemic generation of nitric oxide. *Free Radic. Biol. Med.* 2004, *37*, 395–400.

[26] Zhang, Z., Naughton, D., Winyard, P. G., Benjamin, N. et al., Generation of nitric oxide by a nitrite reductase activity of xanthine oxidase: a potential pathway for nitric oxide formation in the absence of nitric oxide synthase activity. *Biochem. Biophys. Res. Commun.* 1998, *249*, 767–772.

[27] Gladwin, M. T., Cosby, K., Partovi, K., Martyr, S. et al., Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilalates the human circulation. *Circulation* 2003, *108*, 20–21.

[28] Marletta, M. A., Yoon, P. S., Iyengar, R., Leaf, C. D. et al., Macrophage oxidation of L-arginine to nitrite and nitrate: nitric oxide is an intermediate. *Biochemistry* 1988, *27*, 8706–8711.

[29] Leaf, C. D., Wishnok, J. S., Tannenbaum, S. R., L-arginine is a precursor for nitrate biosynthesis in humans. *Biochem. Biophys. Res. Commun.* 1989, *163*, 1032–1037.

[30] Wennmalm, A., Benthin, G., Edlund, A., Jungersten, L. et al., Metabolism and excretion of nitric oxide in humans. An experimental and clinical study. *Circ. Res.* 1993, *73*, 1121–1127.

[31] Van den Brandt, P. A., Willett, W. C., Tannenbaum, S. R., Assessment of dietary nitrate intake by a self-administered questionnaire and by overnight urinary measurement. *Int. J. Epidemiol.* 1989, *18*, 852–857.

[32] Moncada, S., Higgs, A., The L-arginine-nitric oxide pathway. *N. Engl. J. Med.* 1993, *329*, 2002–2012.

[33] Hattori, R., Sase, K., Eizawa, H., Kosuga, K. et al., Structure and function of nitric oxide synthases. *Int. J. Cardiol.* 1994, *47*, S71–S75.

[34] Ohshima, H., Bandaletova, T. Y., Brouet, I., Bartsch, H. et al., Increased nitrosamine and nitrate biosynthesis mediated by nitric oxide synthase induced in hamsters infected with liver fluke (*Opisthorchis viverrini*). *Carcinogenesis* 1994, *15*, 271–275.

[35] Bartsch, H., Ohshima, H., Pignatelli, B., Calmels, S., Endogenously formed *N*-nitroso compounds and nitrosating agents in human cancer etiology. *Pharmacogenetics* 1992, *2*, 272–277.

[36] Schaus, R., Griess' nitrite test in diagnosis of urinary infection. *J. Am. Med. Assoc.* 1956, *161*, 528–529.

[37] Stuehr, D. J., Marletta, M. A., Mammalian nitrate biosynthesis: mouse macrophages produce nitrite and nitrate in response to Escherichia coli lipopolysaccharide. *Proc. Natl. Acad. Sci. USA* 1985, *82*, 7738–7742.

[38] Hofseth, L. J., Hussain, S. P., Wogan, G. N., Harris, C. C., Nitric oxide in cancer and chemoprevention. *Free Radic. Biol. Med.* 2003, *34*, 955–968.

[39] Hussain, S. P., He, P., Subleski, J., Hofseth, L. J. et al., Nitric oxide is a key component in inflammation-accelerated tumorigenesis. *Cancer Res.* 2008, *68*, 7130–7136.

[40] Srivatanakul, P., Ohshima, H., Khlat, M., Parkin, M. et al., *Opisthorchis viverrini* infestation and endogenous nitrosamines as risk factors for cholangiocarcinoma in Thailand. *Int. J. Cancer* 1991, *48*, 821–825.

[41] Sripa, B., Brindley, P. J., Mulvenna, J., Laha, T. et al., The tumorigenic liver fluke *Opisthorchis viverrini*—multiple pathways to cancer. *Trends Parasitol.* 2012, *28*, 395–407.

[42] Jenkins-Holick, D. S., Kaul, T. L., Schistosomiasis. *Urol. Nurs.* 2013, *33*, 163–170.

[43] Mostafa, M. H., Helmi, S., Badawi, A. F., Tricker, A. R. et al., Nitrate, nitrite and volatile *N*-nitroso compounds in the urine of *Schistosoma haematobium* and *Schistosoma mansoni* infected patients. *Carcinogenesis* 1994, *15*, 619–625.

[44] Tricker, A. R., Mostafa, M. H., Spiegelhalder, B., Preussmann, R., Urinary nitrate, nitrite and *N*-nitroso compounds in bladder cancer patients with schistosomiasis (bilharzia). *IARC Sci. Publ.* 1991, *105*, 178–181.

[45] Tricker, A. R., Mostafa, M. H., Spiegelhalder, B., Preussmann, R., Urinary excretion of nitrate, nitrite and *N*-nitroso compounds in Schistosomiasis and bilharzia bladder cancer patients. *Carcinogenesis* 1989, *10*, 547–552.

[46] Calmels, S., Ohshima, H., Crespi, M., Leclerc, H. et al., *N*-nitrosamine formation by microorganisms isolated from human gastric juice and urine: biochemical studies on

bacteria-catalysed nitrosation. *IARC Sci. Publ.* 1987, *84*, 391–395.

[47] Calmels, S., Ohshima, H., Rosenkranz, H., McCoy, E. et al., Biochemical studies on the catalysis of nitrosation by bacteria. *Carcinogenesis* 1987, *8*, 1085–1088.

[48] Leach, S. A., Thompson, M., Hill, M., Bacterially catalysed N-nitrosation reactions and their relative importance in the human stomach. *Carcinogenesis* 1987, *8*, 1907–1912.

[49] Tricker, A. R., Kalble, T., Preussmann, R., Increased urinary nitrosamine excretion in patients with urinary diversions. *Carcinogenesis* 1989, *10*, 2379–2382.

[50] Tricker, A. R., Stickler, D. J., Chawla, J. C., Preussmann, R., Increased urinary nitrosamine excretion in paraplegic patients. *Carcinogenesis* 1991, *12*, 943–946.

[51] Correa, P., Haenszel, W., Cuello, C., Zavala, D. et al., Gastric precancerous process in a high risk population: cross-sectional studies. *Cancer Res.* 1990, *50*, 4731–4736.

[52] Ziebarth, D., Spiegelhalder, B., Bartsch, H., *N*-nitrosation of medicinal drugs catalysed by bacteria from human saliva and gastro-intestinal tract, including *Helicobacter pylori*. *Carcinogenesis* 1997, *18*, 383–389.

[53] Akuta, T., Zaki, M. H., Yoshitake, J., Okamoto, T. et al., Nitrative stress through formation of 8-nitroguanosine: insights into microbial pathogenesis. *Nitric Oxide* 2006, *14*, 101–108.

[54] Dedon, P. C., Tannenbaum, S. R., Reactive nitrogen species in the chemical biology of inflammation. *Arch. Biochem. Biophys.* 2004, *423*, 12–22.

[55] Sawa, T., Ohshima, H., Nitrative DNA damage in inflammation and its possible role in carcinogenesis. *Nitric Oxide* 2006, *14*, 91–100.

[56] Spiegelhalder, B., Eisenbrand, G., Preussmann, R., Influence of dietary nitrate on nitrite content of human saliva: possible relevance to in vivo formation of N-nitroso compounds. *Food Cosmet. Toxicol.* 1976, *14*, 545–548.

[57] Tannenbaum, S. R., Weisman, M., Fett, D., Effect of nitrate intake on nitrite formation in human saliva. *Food Cosmet. Toxicol.* 1976, *14*, 549–552.

[58] Qin, L., Liu, X., Sun, Q., Fan, Z. et al., Sialin (SLC17A5) functions as a nitrate transporter in the plasma membrane. *Proc. Natl. Acad. Sci. USA* 2012, *109*, 13434–13439.

[59] Eisenbrand, G., Spiegelhalder, B., Preussmann, R., Nitrate and nitrite in saliva. *Oncology* 1980, *37*, 227–231.

[60] Eisenbrand, G., in: Nicolai, H. V., Eisenbrand, G., Bozler, G. (Eds.), *The Significance of N-Nitrosation of Drugs*, Gustav Fischer Verlag, Stuttgart, New York 1990, pp. 47–69.

[61] Lundberg, J. O., Weitzberg, E., Lundberg, J. M., Alving, K., Intragastric nitric oxide production in humans: measurements in expelled air. *Gut* 1994, *35*, 1543–1546.

[62] Benjamin, N., O'Driscoll, F., Dougall, H., Duncan, C. et al., Stomach NO synthesis. *Nature* 1994, *368*, 502.

[63] Lundberg, J. O., Weitzberg, E., Cole, J. A., Benjamin, N., Nitrate, bacteria and human health. *Nat. Rev. Microbiol.* 2004, *2*, 593–602.

[64] Dykhuizen, R. S., Fraser, A., McKenzie, H., Golden, M. et al., *Helicobacter pylori* is killed by nitrite under acidic conditions. *Gut* 1998, *42*, 334–337.

[65] Park, A. M., Nagata, K., Sato, E. F., Tamura, T. et al., Mechanism of strong resistance of *Helicobacter pylori* respiration to nitric oxide. *Arch. Biochem. Biophys.* 2003, *411*, 129–135.

[66] Justino, M. C., Ecobichon, C., Fernandes, A. F., Boneca, I. G. et al., *Helicobacter pylori* has an unprecedented nitric oxide detoxifying system. *Antioxid. Redox Signal.* 2012, *17*, 1190–1200.

[67] Govoni, M., Jansson, E. A., Weitzberg, E., Lundberg, J. O., The increase in plasma nitrite after a dietary nitrate load is markedly attenuated by an antibacterial mouthwash. *Nitric Oxide* 2008, *19*, 333–337.

[68] Jansson, E. A., Huang, L., Malkey, R., Govoni, M. et al., A mammalian functional nitrate reductase that regulates nitrite and nitric oxide homeostasis. *Nat. Chem. Biol.* 2008, *4*, 411–417.

[69] Gladwin, M. T., Cosby, K., Partovi, K. S., Patel, R. P. et al., Novel function of human hemoglobin as a nitrite reductase regulates nitric oxide homeostasis and hypoxic vasodilation. *Blood* 2003, *102*, 255a.

[70] Gladwin, M. T., Schechter, A. N., Kim-Shapiro, D. B., Patel, R. P. et al., The emerging biology of the nitrite anion. *Nat. Chem. Biol.* 2005, *1*, 308–314.

[71] van Faassen, E. E., Bahrami, S., Feelisch, M., Hogg, N. et al., Nitrite as regulator of hypoxic signaling in mammalian physiology. *Med. Res. Rev.* 2009, *29*, 683–741.

[72] Shiva, S., Huang, Z., Grubina, R., Sun, J. H. et al., Deoxymyoglobin is a nitrite reductase that generates nitric oxide and regulates mitochondrial respiration. *Circ. Res.* 2007, *100*, 654–661.

[73] Cosby, K., Partovi, K. S., Crawford, J. H., Patel, R. P. et al., Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation. *Nat. Med.* 2003, *9*, 1498–1505.

[74] Nagababu, E., Ramasamy, S., Abernethy, D. R., Rifkind, J. M., Active nitric oxide produced in the red cell under hypoxic conditions by deoxyhemoglobin-mediated nitrite reduction. *J. Biol. Chem.* 2003, *278*, 46349–46356.

[75] Baliga, R. S., Milsom, A. B., Ghosh, S. M., Trinder, S. L. et al., Dietary nitrate ameliorates pulmonary hypertension cytoprotective role for endothelial nitric oxide synthase and xanthine oxidoreductase. *Circulation* 2012, *125*, 2922–2932.

[76] Millar, T. M., Stevens, C. R., Blake, D. R., Xanthine oxidase can generate nitric oxide from nitrate in ischaemia. *Biochem. Soc. Trans.* 1997, *25*, 528S.

[77] Huang, Z., Shiva, S., Kim-Shapiro, D. B., Patel, R. P. et al., Enzymatic function of hemoglobin as a nitrite reductase that produces NO under allosteric control. *J. Clin. Invest.* 2005, *115*, 2099–2107.

[78] Moncada, S., Palmer, R. M., Higgs, E. A., Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.* 1991, *43*, 109–142.

[79] Rees, D. D., Palmer, R. M. J., Moncada, S., Role of endothelium-derived nitric oxide in the regulation of

blood pressure. *Proc. Natl. Acad. Sci. USA* 1989, *86*, 3375–3378.

[80] Ceccatelli, S., Lundberg, J. M., Fahrenkrug, J., Bredt, D. S. et al., Evidence for involvement of nitric oxide in the regulation of hypothalamic portal blood flow. *Neuroscience* 1992, *51*, 769–772.

[81] Palmer, R. M., Ferrige, A. G., Moncada, S., Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 1987, *327*, 524–526.

[82] Radomski, M. W., Palmer, R. M., Moncada, S., Endogenous nitric oxide inhibits human platelet adhesion to vascular endothelium. *Lancet* 1987, *2*, 1057–1058.

[83] Radomski, M. W., Palmer, R. M., Moncada, S., The anti-aggregating properties of vascular endothelium: interactions between prostacyclin and nitric oxide. *Brit. J. Pharmacol.* 1987, *92*, 639–646.

[84] Rand, M. J., Nitrergic transmission: nitric oxide as a mediator of non-adrenergic, non-cholinergic neuro-effector transmission. *Clin. Exp. Pharmacol. Physiol.* 1992, *19*, 147–169.

[85] Bredt, D. S., Snyder, S. H., Nitric oxide, a novel neuronal messenger. *Neuron* 1992, *8*, 3–11.

[86] Garthwaite, J., Boulton, C. L., Nitric oxide signaling in the central nervous system. *Annu. Rev. Physiol.* 1995, *57*, 683–706.

[87] Larsen, F. J., Schiffer, T. A., Weitzberg, E., Lundberg, J. O., Regulation of mitochondrial function and energetics by reactive nitrogen monoxides. *Free Radic. Biol. Med.* 2012, *53*, 1919–1928.

[88] Ceccatelli, S., Hulting, A. L., Zhang, X., Gustafsson, L. et al., Nitric oxide synthase in the rat anterior pituitary gland and the role of nitric oxide in regulation of luteinizing hormone secretion. *Proc. Natl. Acad. Sci. USA* 1993, *90*, 11292–11296.

[89] Langrehr, J. M., Hoffman, R. A., Lancaster, J. R., Simmons, R. L., Nitric oxide—a new endogenous immunomodulator. *Transplantation* 1993, *55*, 1205–1212.

[90] Wei, X. Q., Charles, I. G., Smith, A., Ure, J. et al., Altered immune responses in mice lacking inducible nitric oxide synthase. *Nature* 1995, *375*, 408–411.

[91] Green, S. J., Scheller, L. F., Marletta, M. A., Seguin, M. C. et al., Nitric oxide: cytokine-regulation of nitric oxide in host resistance to intracellular pathogens. *Immunol. Lett.* 1994, *43*, 87–94.

[92] Carnovale, C. E., Ronco, M. T., Role of nitric oxide in liver regeneration. *Ann. Hepatol.* 2012, *11*, 636–647.

[93] Liu, Y., Feng, Q., NOing the heart: role of nitric oxide synthase-3 in heart development. *Differentiation* 2012, *84*, 54–61.

[94] Heinrich, U. R., Helling, K., Nitric oxide—a versatile key player in cochlear function and hearing disorders. *Nitric Oxide* 2012, *27*, 106–116.

[95] Steinberg, A., Nilsson Remahl, A. I., Role of nitric oxide in cluster headache. *Curr. Pain Headache Rep.* 2012, *16*, 185–190.

[96] Grasemann, H., Ratjen, F., Nitric oxide and L-arginine deficiency in cystic fibrosis. *Curr. Pharm. Des.* 2012, *18*, 726–736.

[97] Kroncke, K. D., Fehsel, K., Suschek, C., Kolb-Bachofen, V., Inducible nitric oxide synthase-derived nitric oxide in gene regulation, cell death and cell survival. *Int. Immunopharmacol.* 2001, *1*, 1407–1420.

[98] Messmer, U. K., Brune, B., Nitric oxide-induced apoptosis: p53-dependent and p53-independent signalling pathways. *Biochem. J.* 1996, *319*, 299–305.

[99] Burke, A. J., Sullivan, F. J., Giles, F. J., Glynn, S. A., The yin and yang of nitric oxide in cancer progression. *Carcinogenesis* 2013, *34*, 503–512.

[100] Mocellin, S., Nitric oxide: cancer target or anticancer agent? *Curr. Cancer Drug Targets* 2009, *9*, 214–236.

[101] Xu, W., Liu, L. Z., Loizidou, M., Ahmed, M. et al., The role of nitric oxide in cancer. *Cell Res.* 2002, *12*, 311–320.

[102] Morcos, E., Carlsson, S., Weitzberg, E., Wiklund, N. P. et al., Inhibition of cancer cell replication by inorganic nitrite. *Nutr. Cancer* 2010, *62*, 501–504.

[103] Dejam, A., Hunter, C. J., Gladwin, M. T., Effects of dietary nitrate on blood pressure. *N. Engl. J. Med.* 2007, *356*, 1590–1590.

[104] Kapil, V., Milsom, A. B., Okorie, M., Maleki-Toyserkani, S. et al., Inorganic nitrate supplementation lowers blood pressure in humans: role for nitrite-derived NO. *Hypertension* 2010, *56*, 274-281.

[105] Larsen, F. J., Ekblom, B., Sahlin, K., Lundberg, J. O. et al., Effects of dietary nitrate on blood pressure in healthy volunteers. *N. Engl. J. Med.* 2006, *355*, 2792–2793.

[106] Gladwin, M. T., Shelhamer, J. H., Schechter, A. N., Pease-Fye, M. E. et al., Role of circulating nitrite and S-nitrosohemoglobin in the regulation of regional blood flow in humans. *Proc. Natl. Acad. Sci. USA* 2000, *97*, 11482–11487.

[107] Lundberg, J. O., Weitzberg, E., NO generation from nitrite and its role in vascular control. *Arterioscler. Thromb. Vasc. Biol.* 2005, *25*, 915–922.

[108] Maher, A. R., Thomas, P., Anderson, S., Abozguia, K. et al., Intra-arterial nitrite causes an enduring vasodilation in man. *J. Am. Coll. Cardiol.* 2008, *51*, A284.

[109] Velmurugan, S., Pearl, V., Davies, S., Bahra, M. et al., Inorganic nitrate supplementation attenuates platelet reactivity in healthy male volunteers. *Nitric Oxide* 2012, *27*, S27–S28.

[110] Jung, K. H., Chu, K., Ko, S. Y., Lee, S. T. et al., Early intravenous infusion of sodium nitrite protects brain against in vivo ischemia-reperfusion injury. *Stroke* 2006, *37*, 2744–2750.

[111] Webb, A., Bond, R., McLean, P., Uppal, R. et al., Reduction of nitrite to nitric oxide during ischemia protects against myocardial ischemia-reperfusion damage. *Proc. Natl. Acad. Sci. USA* 2004, *101*, 13683–13688.

[112] Duranski, M. R., Greer, J. J., Dejam, A., Jaganmohan, S. et al., Cytoprotective effects of nitrite during in vivo ischemia-reperfusion of the heart and liver. *J. Clin. Invest.* 2005, *115*, 1232–1240.

[113] Tripatara, P., Patel, N. S. A., Webb, A., Rathod, K. et al., Nitrite-derived nitric oxide protects the rat kidney against ischemia/reperfusion injury in vivo: role for xanthine oxidoreductase. *J. Am. Soc. Nephrol.* 2007, *18*, 570–580.

[114] Hendgen-Cotta, U. B., Luedike, P., Totzeck, M., Kropp, M. et al., Dietary nitrate supplementation improves revascularization in chronic ischemia. *Circulation* 2012, *126*, 1983–1992.

[115] Dejam, A., Hunter, C. J., Tremonti, C., Pluta, R. M. et al., Nitrite infusion in humans and nonhuman primates: endocrine effects, pharmacokinetics, and tolerance formation. *Circulation* 2007, *116*, 1821–1831.

[116] Heiss, C., Meyer, C., Totzeck, M., Hendgen-Cotta, U. B. et al., Dietary inorganic nitrate mobilizes circulating angiogenic cells. *Free Radic. Biol. Med.* 2012, *52*, 1767–1772.

[117] Webb, A. J., Patel, N., Loukogeorgakis, S., Okorie, M. et al., Acute blood pressure lowering, vasoprotective, and antiplatelet properties of dietary nitrate via bioconversion to nitrite. *Hypertension* 2008, *51*, 784–790.

[118] Aboud, Z., Misra, S., Warner, T., Miall, P. et al., The enterosalivary bioconversion of nitrate to nitrite underlies the blood pressure (BP) lowering and anti-platelet effects of a dietary nitrate load. *Brit. J. Clin. Pharmacol.* 2008, *65*, 999.

[119] Richardson, G., Hicks, S. L., O'Byrne, S., Frost, M. T. et al., The ingestion of inorganic nitrate increases gastric S-nitrosothiol levels and inhibits platelet function in humans. *Nitric Oxide* 2002, *7*, 24–29.

[120] Carlstrom, M., Persson, A. E., Larsson, E., Hezel, M. et al., Dietary nitrate attenuates oxidative stress, prevents cardiac and renal injuries, and reduces blood pressure in salt-induced hypertension. *Cardiovasc. Res.* 2011, *89*, 574–585.

[121] Bjorne, H. H., Petersson, J., Phillipson, M., Weitzberg, E. et al., Nitrite in saliva increases gastric mucosal blood flow and mucus thickness. *J. Clin. Invest.* 2004, *113*, 106–114.

[122] Petersson, J., Phillipson, M., Jansson, E. A., Patzak, A. et al., Dietary nitrate increases gastric mucosal blood flow and mucosal defense. *Am. J. Physiol.* 2007, *292*, G718–G724.

[123] Carlstrom, M., Larsen, F. J., Nystrom, T., Hezel, M. et al., Dietary inorganic nitrate reverses features of metabolic syndrome in endothelial nitric oxide synthase-deficient mice. *Proc. Natl. Acad. Sci. USA* 2010, *107*, 17716–17720.

[124] Fernandez, M. L., Ruiz, R., Gonzalez, M. A., Ramirez-Lorca, R. et al., Association of NOS3 gene with metabolic syndrome in hypertensive patients. *Thromb. Haemost.* 2004, *92*, 413–418.

[125] Huang, P. L., eNOS, metabolic syndrome and cardiovascular disease. *Trends Endocrinol. Metab.* 2009, *20*, 295–302.

[126] Moncada, S., Palmer, R. M., Higgs, E. A., The discovery of nitric oxide as the endogenous nitrovasodilator. *Hypertension* 1988, *12*, 365–372.

[127] Modin, A., Bjorne, H., Herulf, M., Alving, K. et al., Nitrite-derived nitric oxide: a possible mediator of 'acidic-metabolic' vasodilation. *Acta Physiol. Scand.* 2001, *171*, 9–16.

[128] Rodriguez, J., Maloney, R. E., Rassaf, T., Bryan, N. S. et al., Chemical nature of nitric oxide storage forms in rat vascular tissue. *Proc. Natl. Acad. Sci. USA* 2003, *100*, 336–341.

[129] Webb, A. J., Milsom, A. B., Rathod, K. S., Chu, W. L. et al., Mechanisms underlying erythrocyte and endothelial nitrite reduction to nitric oxide in hypoxia: role for xanthine oxidoreductase and endothelial nitric oxide synthase. *Circ. Res.* 2008, *103*, 957–964.

[130] Petersson, J., Carlstrom, M., Schreiber, O., Phillipson, M. et al., Gastroprotective and blood pressure lowering effects of dietary nitrate are abolished by an antiseptic mouthwash. *Free Radic. Biol. Med.* 2009, *46*, 1068–1075.

[131] Kapil, V., Haydar, S. M., Pearl, V., Lundberg, J. O. et al., Physiological role for nitrate-reducing oral bacteria in blood pressure control. *Free Radic. Biol. Med.* 2013, *55*, 93–100.

[132] Zweier, J. L., Wang, P., Kuppusamy, P., Direct measurement of nitric oxide generation in the ischemic heart using electron paramagnetic resonance spectroscopy. *J. Biol. Chem.* 1995, *270*, 304–307.

[133] Zweier, J. L., Wang, P., Samouilov, A., Kuppusamy, P., Enzyme-independent formation of nitric oxide in biological tissues. *Nat. Med.* 1995, *1*, 804–809.

[134] Duranski, M. R., Greer, J. J. M., Dejam, A., Jaganmohan, S. et al., Cytoprotective effects of nitrite during in vivo ischemia-reperfusion of the heart and liver. *J. Clin. Invest.* 2005, *115*, 1232–1240.

[135] Kapil, V., Okorie, M., Akram, F., Rehman, F. et al., Oral inorganic nitrate lowers blood pressure and protects against endothelial ischaemia-reperfusion injury in humans. *Nitric Oxide* 2009, *20*, S37.

[136] Okorie, M., Akram, F., Loukogeorgakis, S., Webb, A. et al., Oral inorganic nitrate protects against endothelial ischaemia-reperfusion injury in humans. *Basic Clin. Pharmacol.* 2009, *105*, 26.

[137] Ignarro, L. J., Buga, G. M., Wood, K. S., Byrns, R. E. et al., Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc. Natl. Acad. Sci. USA* 1987, *84*, 9265–9269.

[138] Khan, M. T., Furchgott, R. F., Similarities of behavior of nitric oxide (NO) and endothelium-derived relaxing factor in a perfusion cascade bioassay system. *Fed. Proc.* 1987, *46*, 385A.

[139] Srihirun, S., Sriwantana, T., Unchern, S., Kittikool, D. et al., Platelet inhibition by nitrite is dependent on erythrocytes and deoxygenation. *PLoS One* 2012, *7*, e30380.

[140] Joshipura, K. J., Ascherio, A., Manson, J. E., Stampfer, M. J. et al., Fruit and vegetable intake in relation to risk of ischemic stroke. *J. Am. Med. Assoc.* 1999, *282*, 1233–1239.

[141] Joshipura, K. J., Hu, F. B., Manson, J. E., Stampfer, M. J. et al., The effect of fruit and vegetable intake on risk for coronary heart disease. *Ann. Intern. Med.* 2001, *134*, 1106–1114.

[142] Hung, H. C., Joshipura, K. J., Jiang, R., Hu, F. B. et al., Fruit and vegetable intake and risk of major chronic disease. *J. Natl. Cancer Inst.* 2004, *96*, 1577–1584.

[143] Lundberg, J. O., Carlstrom, M., Larsen, F. J., Weitzberg, E., Roles of dietary inorganic nitrate in cardiovascular health and disease. *Cardiovasc. Res.* 2011, *89*, 525–532.

[144] Carter, P., Gray, L. J., Troughton, J., Khunti, K. et al., Fruit and vegetable intake and incidence of type 2 diabetes mellitus: systematic review and meta-analysis. *Brit. Med. J.* 2010, *341*, c4229.

[145] Webb, A. J., Patel, N., Loukogeorgakis, S., Okorie, M. et al., Acute blood pressure (BP) lowering and vasoprotective effects of beetroot juice: relationship to nitrite derived from dietary nitrate. *J. Hum. Hypertens.* 2007, *21*, 834–835.

[146] Kapil, V., Inorganic nitrate supplementation lowers blood pressure in humans: role for nitrite-derived NO (vol 56, 274, 2010). *Hypertension* 2010, *56*, e37–e39.

[147] Siervo, M., Lara, J., Ogbonmwan, I., Mathers, J. C., Inorganic nitrate and beetroot juice supplementation reduces blood pressure in adults: a systematic review and meta-analysis. *J. Nutr.* 2013, *143*, 818–826.

[148] Sobko, T., Marcus, C., Govoni, M., Kamiya, S., Dietary nitrate in Japanese traditional foods lowers diastolic blood pressure in healthy volunteers. *Nitric Oxide* 2010, *22*, 136–140.

[149] Bailey, S. J., Fulford, J., Vanhatalo, A., Winyard, P. G. et al., Dietary nitrate supplementation enhances muscle contractile efficiency during knee-extensor exercise in humans. *J. Appl. Physiol.* 2010, *109*, 135–148.

[150] Bailey, S. J., Winyard, P., Vanhatalo, A., Blackwell, J. R. et al., Dietary nitrate supplementation reduces the O_2 cost of low-intensity exercise and enhances tolerance to high-intensity exercise in humans. *J. Appl. Physiol.* 2009, *107*, 1144–1155.

[151] Lansley, K. E., Winyard, P. G., Fulford, J., Vanhatalo, A. et al., Dietary nitrate supplementation reduces the O_2 cost of walking and running: a placebo-controlled study. *J. Appl. Physiol.* 2011, *110*, 591–600.

[152] Vanhatalo, A., Bailey, S. J., Blackwell, J. R., DiMenna, F. J. et al., Acute and chronic effects of dietary nitrate supplementation on blood pressure and the physiological responses to moderate-intensity and incremental exercise. *Am. J. Physiol.* 2010, *299*, R1121–R1131.

[153] Bailey, S. J., Fulford, J., Vanhatalo, A., Winyard, P. G. et al., Dietary nitrate supplementation enhances muscle contractile efficiency during knee-extensor exercise in humans. *J. Appl. Physiol.* 2010, *109*, 135–148.

[154] Larsen, F. J., Schiffer, T. A., Borniquel, S., Sahlin, K. et al., Dietary inorganic nitrate improves mitochondrial efficiency in humans. *Cell Metab.* 2011, *13*, 149–159.

[155] Kenjale, A. A., Ham, K. L., Stabler, T., Robbins, J. L. et al., Dietary nitrate supplementation enhances exercise performance in peripheral arterial disease. *J. Appl. Physiol.* 2011, *110*, 1582–1591.

[156] Collins, R., Peto, R., MacMahon, S., Hebert, P. et al., Blood pressure, stroke, and coronary heart disease. Part 2, short-term reductions in blood pressure: overview of randomised drug trials in their epidemiological context. *Lancet* 1990, *335*, 827–838.

[157] Lv, J., Neal, B., Ehteshami, P., Ninomiya, T. et al., Effects of intensive blood pressure lowering on cardiovascular and renal outcomes: a systematic review and meta-analysis. *PLoS Med.* 2012, *9*, e1001293.

[158] Barter, P. J., Caulfield, M., Eriksson, M., Grundy, S. M. et al., Effects of torcetrapib in patients at high risk for coronary events. *N. Engl. J. Med.* 2007, *357*, 2109–2122.

[159] Ivankovic, S., Teratogenic and carcinogenic effects of some chemicals during perinatal life in rats, Syrian golden hamsters, and minipigs. *Natl. Cancer Inst. Monog.* 1979, *51*, 103–115.

[160] Platzek, T., Bochert, G., Rahm, U., Embryotoxicity induced by alkylating agents. Teratogenicity of acetoxymethylmethylnitrosamine: dose-response relationship, application route dependency and phase specificity. *Arch. Toxicol.* 1983, *52*, 45–69.

[161] Nagao, T., Morita, Y., Ishizuka, Y., Wada, A. et al., Induction of fetal malformations after treatment of mouse embryos with methylnitrosourea at the preimplantation stages. *Teratog. Carcinog. Mutagen.* 1991, *11*, 1–10.

[162] Lijinsky, W., *Chemistry and Biology of N-Nitroso Compounds*. University Press, Cambridge 1992.

[163] Voss, C., Herrmann, I., Hartmann, K., Zuhlke, H. et al., Diabetogenic effects of *N*-nitrosomethylurea in rats. *Exp. Clin. Endocrinol.* 1988, *92*, 25–31.

[164] Brender, J. D., Werler, M. M., Kelley, K. E., Vuong, A. M. et al., Nitrosatable drug exposure during early pregnancy and neural tube defects in offspring: National Birth Defects Prevention Study. *Am. J. Epidemiol.* 2011, *174*, 1286–1295.

[165] Brender, J. D., Werler, M. M., Shinde, M. U., Vuong, A. M. et al., Nitrosatable drug exposure during the first trimester of pregnancy and selected congenital malformations. *Birth Defects Res. A Clin. Mol. Teratol.* 2012, *94*, 701–713.

[166] Huber, J., Zheng, Q., Sharkey, J., Brender, J. et al., Dietary nitrates, nitrites and nitrosatable compounds and neural tube defects, oral clefts and limb deficiencies. *Am. J. Epidemiol.* 2011, *173*, S297–S297.

[167] Huber, J. C., Jr., Brender, J. D., Zheng, Q., Sharkey, J. R. et al., Maternal dietary intake of nitrates, nitrites and nitrosamines and selected birth defects in offspring: a case-control study. *Nutr. J.* 2013, *12*, 34.

[168] Shinde, M. U., Vuong, A. M., Brender, J. D., Werler, M. M. et al., Prenatal exposure to nitrosatable drugs, vitamin C, and risk of selected birth defects. *Birth Defects Res. A Clin. Mol. Teratol.* 2013, *97*, 515–531.

[169] Brender, J. D., Olive, J. M., Felkner, M., Suarez, L. et al., Dietary nitrites and nitrates, nitrosatable drugs, and neural tube defects. *Epidemiology* 2004, *15*, 330–336.

[170] Li, Z. W., Zhang, L., Ye, R. W., Liu, J. M. et al., Maternal periconceptional consumption of pickled vegetables and risk of neural tube defects in offspring. *Chin. Med. J.* 2011, *124*, 1629–1633.

[171] McKean-Cowdin, R., Pogoda, J. M., Lijinsky, W., Holly, E. A. et al., Maternal prenatal exposure to nitrosatable drugs and childhood brain tumours. *Int. J. Epidemiol.* 2003, *32*, 211–217.

[172] Croen, L. A., Todoroff, K., Shaw, G. M., Maternal exposure to nitrate from drinking water and diet and risk for neural tube defects. *Am. J. Epidemiol.* 2001, *153*, 325–331.

[173] Sander, J., Burkle, G., Induction of malignant tumors in rats by simultaneous feeding of nitrite and secondary amines. *Z. Krebsforsch.* 1969, *73*, 54–66.

[174] Ohshima, H., Bartsch, H., Quantitative estimation of endogenous nitrosation in humans by monitoring *N*-nitrosoproline excreted in the urine. *Cancer Res.* 1981, *41*, 3658–3662.

[175] Ohshima, H., Bereziat, J. C., Bartsch, H., Measurement of endogenous *N*-nitrosation in rats and humans by monitoring urinary and faecal excretion of *N*-nitrosamino acids. *IARC Sci. Publ.* 1982, *41*, 397–411.

[176] Janzowski, C., Hemm, I., Eisenbrand, G., in: Wichmann, H.-E., Schlipköter, H. W., Fülgraff, G. (Eds.), *Handbuch der Umweltmedizin*, ecomed Verlagsgesellschaft, Landsberg 2000.

[177] World Cancer Research Fund/American Institute for Cancer Research. Systematic Literature Review Continuous Update Project Report: The Associations between Food, Nutrition and Physical Activity and the Risk of Colorectal Cancer, 2010. Available from: http://www.wcrf.org/sites/default/files/SLR_colorectal_cancer_2010.pdf.

[178] Ridd, J. H., Nitrosation, diazotisation, and deamination. *Q. Rev. Chem. Soc.* 1961, *15*, 418–441.

[179] Mirvish, S. S., Formation of *N*-nitroso compounds: Chemistry, kinetics, and *in vivo* occurrence. *Toxicol. Appl. Pharm.* 1975, *31*, 325–351.

[180] Mirvish, S. S., Kinetics of dimethylamine nitrosation in relation to nitrosamine carcinogenesis. *J. Natl. Cancer Inst.* 1970, *44*, 633–639.

[181] Keefer, L. K., Roller, P. P., *N*-nitrosation by nitrite ion in neutral and basic medium. *Science* 1973, *181*, 1245–1247.

[182] Loeppky, R. N., Bao, Y. T., Bae, J. Y., Yu, L. et al., in: Loeppky, R. N., Michejda, C. J. (Eds.), *Nitrosamines and Related N-Nitroso Compounds: Chemistry and Biochemistry*, American Chemical Society, Washington, DC 1994, pp. 52–65.

[183] Abraham, S. K., Khandelwal, N., Ascorbic acid and dietary polyphenol combinations protect against genotoxic damage induced in mice by endogenous nitrosation. *Mutat. Res.* 2013, *757*, 167–172.

[184] Khandelwal, N., Abraham, S. K., Intake of anthocyanidins pelargonidin and cyanidin reduces genotoxic stress in mice induced by diepoxybutane, urethane and endogenous nitrosation. *Environ. Toxicol. Pharmacol.* 2014, *37*, 837–843.

[185] Conforti, F., Menichini, F., Phenolic compounds from plants as nitric oxide production inhibitors. *Curr. Med. Chem.* 2011, *18*, 1137–1145.

[186] Bellander, T., in: Nicolai, H. V., Eisenbrand, G., Bozler, G. (Eds.), *The Significance of N-Nitrosation of Drugs*, Gustav Fischer Verlag, Stuttgart, New York 1990, pp. 213–233.

[187] Habermeyer, M., Eisenbrand, G., in: Stadler, S., Lineback, D. (Eds.), *Process-Induced Food Toxicants and Health Risks*, John Wiley & Sons, Hoboken, New Jersey 2008.

[188] Tricker, A. R., Kubacki, S. J., Review of the occurrence and formation of nonvolatile N-nitroso compounds in foods. *Food Addit. Contam.* 1992, *9*, 39–69.

[189] EU Scientific Committee on Consumer Safety (SCCS), Opinion on Nitrosamines and Secondary Amines in Cosmetic Products (SCCS/1458/11), 2012. Available from: http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_072.pdf.

[190] EU Scientific Committee on Consumer Safety (SCCS), Opinion on NDELA in Cosmetic Products and Nitrosamines in Balloons (SCCS/1486/12), 2012. Available from: http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_100.pdf.

[191] Lijinsky, W., in: Nicolai, H. V., Eisenbrand, G., Bozler, G. (Eds.), *The Significance of N-Nitrosation of Drugs*, Gustav Fischer Verlag, Stuttgart, New York 1990, pp. 93–107.

[192] Vermeer, I. T. M., Pachen, D. M. F. A., Dallinga, J. W., Kleinjans, J. C. S. et al., Volatile *N*-nitrosamine formation after intake of nitrate at the ADI level in combination with an amine-rich diet. *Environ. Health Perspect.* 1998, *106*, 459–463.

[193] Kuhnle, G. G. C., Story, G. W., Reda, T., Mani, A. R. et al., Diet-induced endogenous formation of nitroso compounds in the GI tract. *Free Radic. Biol. Med.* 2007, *43*, 1040–1047.

[194] Tricker, A. R., Kumar, R., Siddiqi, M., Khuroo, M. S. et al., Endogenous formation of *N*-nitrosamines from piperazine and their urinary excretion following antihelminthic treatment with piperazine citrate. *Carcinogenesis* 1991, *12*, 1595–1599.

[195] Lijinsky, W., Taylor, H. W., Snyder, C., Nettesheim, P., Malignant tumors of liver and lung in rats fed aminopyrine or heptamethyleneimine together with nitrite. *Nature* 1973, *244*, 176–177.

[196] Spiegelhalder, B., in: Nicolai, H. V., Eisenbrand, G., Bozler, G. (Eds.), *The Significance of N-Nitrosation of Drugs*, Gustav Fischer Verlag, Stuttgart, New York 1990, pp. 199–212.

[197] Spiegelhalder, B., Preussmann, R., *In vivo* nitrosation of amidopyrine in humans: use of "ethanol effect" for biological monitoring of *N*-nitrosodimethylamine in urine. *Carcinogenesis* 1985, *6*, 545–548.

[198] Adam, B., Schlag, P., Friedl, P., Preussmann, R. et al., Proline is not useful as a chemical probe to measure nitrosation in the gastrointestinal tract of patients with gastric disorders characterised by anacidic conditions. *Gut* 1989, *30*, 1068–1075.

[199] Carmella, S. G., Borukhova, A., Desai, D., Hecht, S. S., Evidence for endogenous formation of tobacco-specific nitrosamines in rats treated with tobacco alkaloids and sodium nitrite. *Carcinogenesis* 1997, *18*, 587–592.

[200] Hecht, S. S., Chen, C.-H. B., Ornaf, R. M., Jacobs, E. et al., Chemical studies on tobacco smoke. 52. Reaction of nicotine and sodium nitrite: formation of nitrosamines and fragmentation of the pyrrolidine ring. *J. Org. Chem.* 1978, *43*, 72–76.

[201] Mirvish, S. S., Sams, J., Hecht, S. S., Kinetics of nornicotine and anabasine nitrosation in relation to *N'*-nitrosonornicotine occurrence in tobacco and to tobacco-induced cancer. *J. Natl. Cancer Inst.* 1977, *59*, 1211–1213.

[202] Stepanov, I., Carmella, S. G., Briggs, A., Hertsgaard, L. et al., Presence of the carcinogen *N'*-nitrosonornicotine in the urine of some users of oral nicotine replacement therapy products. *Cancer Res.* 2009, *69*, 8236–8240.

[203] Stepanov, I., Carmella, S. G., Han, S., Pinto, A. et al., Evidence for endogenous formation of *N'*-nitrosonornicotine in some long-term nicotine patch users. *Nicotine Tob. Res.* 2009, *11*, 99–105.

[204] Stepanov, I., Sebero, E., Wang, R., Gao, Y. T. et al., Tobacco-specific *N*-nitrosamine exposures and cancer risk in the Shanghai cohort study: remarkable coherence with rat tumor sites. *Int. J. Cancer* 2014, *134*, 2278–2283.

[205] Yuan, J. M., Knezevich, A. D., Wang, R., Gao, Y. T. et al., Urinary levels of the tobacco-specific carcinogen *N'*-nitrosonornicotine and its glucuronide are strongly associated with esophageal cancer risk in smokers. *Carcinogenesis* 2011, *32*, 1366–1371.

[206] Hecht, S. S., Biochemistry, biology, and carcinogenicity of tobacco-specific *N*-nitrosamines. *Chem. Res. Toxicol.* 1998, *11*, 559–603.

[207] *International Agency for Research on Cancer (IARC), Monographs on the Evaluation of the Carcinogenic Risks to Humans, Vol. 89, Smokeless Tobacco and Some Tobacco-specific N-Nitrosamines*, World Health Organization, Lyon, France 2007.

[208] Puju, S., Shuker, D. E. G., Bishop, W. W., Falchuk, K. R. et al., Mutagenicity of *N*-nitroso bile acid conjugates in *Salmonella typhimurium* and diploid human lymphoblasts. *Cancer Res.* 1982, *42*, 2601–2604.

[209] Busby, W. F., Shuker, D. E. G., Charnley, G., Newberne, P. M. et al., Carcinogenicity in rats of the nitrosated bile acid conjugates *N*-nitrosoglycocholic acid and *N*-nitrosotaurocholic acid. *Cancer Res.* 1985, *45*, 1367–1371.

[210] Shuker, D. E. G., Margison, G. P., Nitrosated glycine derivatives as a potential source of O6-methylguanine in DNA. *Cancer Res.* 1997, *57*, 366–369.

[211] Senthong, P., Millington, C. L., Wilkinson, O. J., Marriott, A. S. et al., The nitrosated bile acid DNA lesion O^6-carboxymethylguanine is a substrate for the human DNA repair protein O^6-methylguanine-DNA methyltransferase. *Nucleic Acids Res.* 2013, *41*, 3047–3055.

[212] Gottschalg, E., Scott, G. B., Burns, P. A., Shuker, D. E. G., Potassium diazoacetate-induced *p53* mutations *in vitro* in relation to formation of O^6-carboxymethyl- and O^6-methyl-2′-deoxyguanosine DNA adducts: relevance for gastrointestinal cancer. *Carcinogenesis* 2007, *28*, 356–362.

[213] Harrison, K. L., Fairhurst, N., Challis, B. C., Shuker, D. E. G., Synthesis, characterization, and immunochemical detection of O^6-(carboxymethyl)-2′-deoxyguanosine: A DNA adduct formed by nitrosated glycine derivatives. *Chem. Res. Toxicol.* 1997, *10*, 652–659.

[214] Cupid, B. C., Zeng, Z. T., Singh, R., Shuker, D. E. G., Detection of O^6-carboxymethyl-2′-deoxyguanosine in DNA following reaction of nitric oxide with glycine and in human blood DNA using a quantitative immunoslot blot assay. *Chem. Res. Toxicol.* 2004, *17*, 294–300.

[215] Lewin, M. H., Bailey, N., Bandaletova, T., Bowman, R. et al., Red meat enhances the colonic formation of the DNA adduct O^6-carboxymethyl guanine: implications for colorectal cancer. *Cancer Res.* 2006, *66*, 1859–1865.

[216] Vanden Bussche, J., Moore, S. A., Pasmans, F., Kuhnle, G. G. C. et al., An approach based on ultra-high pressure liquid chromatography-tandem mass spectrometry to quantify O^6-methyl and O^6-carboxymethylguanine DNA adducts in intestinal cell lines. *J. Chromatogr. A* 2012, *1257*, 25–33.

[217] Bulay, O., Mirvish, S. S., Garcia, H., Pelfrene, A. F. et al., Carcinogenicity test of 6 nitrosamides and a nitrosocyanamide administered orally to rats. *J. Natl. Cancer Inst.* 1979, *62*, 1523–1528.

[218] Cheng, G., Wang, M., Villalta, P. W., Hecht, S. S., Detection of 7-(2′-carboxyethyl)guanine but not 7-carboxymethylguanine in human liver DNA. *Chem. Res. Toxicol.* 2010, *23*, 1089–1096.

[219] Krul, C. A., Zeilmaker, M. J., Schothorst, R. C., Havenaar, R., Intragastric formation and modulation of N-nitrosodimethylamine in a dynamic in vitro gastrointestinal model under human physiological conditions. *Food Chem. Toxicol.* 2004, *42*, 51–63.

[220] Zeilmaker, M. J., Bakker, M. I., Schothorst, R., Slob, W., Risk assessment of *N*-nitrosodimethylamine formed endogenously after fish-with-vegetable meals. *Toxicol. Sci.* 2010, *116*, 323–335.

[221] Pfundstein, B., Tricker, A. R., Theobald, E., Spiegelhalder, B. et al., Mean daily intake of primary and secondary amines from foods and beverages in West Germany in 1989-1990. *Food Chem. Toxicol.* 1991, *29*, 733–739.

[222] Engemann, A., Focke, C., Humpf, H. U., Intestinal formation of *N*-nitroso compounds in the pig cecum model. *J. Agric. Food Chem.* 2013, *61*, 998–1005.

[223] Magee, P. N., Barnes, J. M., The production of malignant primary hepatic tumours in the rat by feeding dimethylnitrosamine. *Brit. J. Cancer* 1956, *10*, 114–122.

[224] Schmahl, D., Preussmann, R., Hamperl, H., Leberkrebserzeugende Wirkung von Diäthylnitrosamin nach oraler Gabe bei Ratten. *Naturwissenschaften* 1960, *47*, 89.

[225] Druckrey, H., Preussmann, R., Ivankovic, S., Schmahl, D., [Organotropic carcinogenic effects of 65 various N-nitroso-compounds on BD rats]. *Z. Krebsforsch.* 1967, *69*, 103–201.

[226] Berger, M. R., Schmahl, D., Edler, L., Implications of the carcinogenic hazard of low doses of three hepatocarcinogenic *N*-nitrosamines. *Jpn. J. Cancer Res.* 1990, *81*, 598–606.

[227] Berger, M. R., Schmahl, D., Zerban, H., Combination experiments with very low doses of three genotoxic *N*-nitrosamines with similar organotropic carcinogenicity in rats. *Carcinogenesis* 1987, *8*, 1635–1643.

[228] Peto, R., Gray, R., Brantom, P., Grasso, P., Dose and time relationships for tumor induction in the liver and esophagus of 4080 inbred rats by chronic ingestion of *N*-nitrosodiethylamine or *N*-nitrosodimethylamine. *Cancer Res.* 1991, *51*, 6452–6469.

[229] Peto, R., Gray, R., Brantom, P., Grasso, P., Effects on 4080 rats of chronic ingestion of *N*-nitrosodiethylamine or *N*-nitrosodimethylamine: a detailed dose-response study. *Cancer Res.* 1991, *51*, 6415–6451.

[230] Hecht, S. S., Trushin, N., Castonguay, A., Rivenson, A., Comparative tumorigenicity and DNA methylation in F344 rats

by 4-(methylNitrosamino)-1-(3-pyridyl)-1-butanone and *N*-nitrosodimethylamine. *Cancer Res.* 1986, *46*, 498–502.

[231] Preussmann, R., in: Nicolai, H. V., Eisenbrand, G., Bozler, G. (Eds.), *The Significance of N-Nitrosation of Drugs*, Gustav Fischer Verlag, Stuttgart, New York 1990, pp. 3–18.

[232] Gombar, C. T., Harrington, G. W., Pylypiw, H. M., Jr., Anderson, L. M. et al., Interspecies scaling of the pharmacokinetics of *N*-nitrosodimethylamine. *Cancer Res.* 1990, *50*, 4366–4370.

[233] Gombar, C. T., Harrington, G. W., Pylypiw, H. M., Jr., Bevill, R. F. et al., Pharmacokinetics of *N*-nitrosodimethylamine in swine. *Carcinogenesis* 1988, *9*, 1351–1354.

[234] Gombar, C. T., Pylypiw, H. M., Jr., Harrington, G. W., Pharmacokinetics of *N*-nitrosodimethylamine in beagles. *Cancer Res.* 1987, *47*, 343–347.

[235] Mico, B. A., Swagzdis, J. E., Hu, H. S., Keefer, L. K. et al., Low-dose *in vivo* pharmacokinetic and deuterium isotope effect studies of *N*-nitrosodimethylamine in rats. *Cancer Res.* 1985, *45*, 6280–6285.

[236] Streeter, A. J., Nims, R. W., Anderson, L. M., Heur, Y. H. et al., Single-dose toxicokinetics of *N*-nitrosomethylethylamine and *N*-nitrosomethyl (2,2,2-trideuterioethyl)amine in the rat. *Arch. Toxicol.* 1990, *64*, 109–115.

[237] Lijinsky, W., Structure-activity relations in carcinogenesis by *N*-nitroso compounds. *Cancer Metastasis Rev.* 1987, *6*, 301–356.

[238] O'Brien, J., Renwick, A. G., Constable, A., Dybing, E. et al., Approaches to the risk assessment of genotoxic carcinogens in food: a critical appraisal. *Food Chem. Toxicol.* 2006, *44*, 1613–1635.

[239] Benford, D., Bolger, P. M., Carthew, P., Coulet, M. et al., Application of the margin of exposure (MOE) approach to substances in food that are genotoxic and carcinogenic. *Food Chem. Toxicol.* 2010, *48*(Suppl 1), S2–S24.

[240] European Food Safety Authority (EFSA), Opinion of the Scientific Committee on a request from EFSA related to a harmonised approach for risk assessment of substances which are both genotoxic and carcinogenic. *EFSA J.* 2005, *3*, 31.

[241] Maekawa, A., Ogiu, T., Onodera, H., Furuta, K. et al., Carcinogenicity studies of sodium nitrite and sodium nitrate in F-344 rats. *Food Chem. Toxicol.* 1982, *20*, 25–33.

[242] *International Agency for Research on Cancer (IARC), Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 94, Ingested Nitrate and Nitrite, and Cyanobacterial Peptide Toxins*, World Health Organization, Lyon, France 2010.

[243] U.S. National Toxicology Program (NTP), NTP Technical Report on the toxicology and carcinogenesis studies of sodium nitrite in F344/N rats and B6C3F$_1$ mice, National Institutes of Health, 2001. Available from: http://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr495.pdf.

[244] Hebels, D. G., Brauers, K. J., van Herwijnen, M. H., Georgiadis, P. A. et al., Time-series analysis of gene expression profiles induced by nitrosamides and nitrosamines elucidates modes of action underlying their genotoxicity in human colon cells. *Toxicol. Lett.* 2011, *207*, 232–241.

[245] Hebels, D. G., Jennen, D. G., van Herwijnen, M. H., Moonen, E. J. et al., Whole-genome gene expression modifications associated with nitrosamine exposure and micronucleus frequency in human blood cells. *Mutagenesis* 2011, *26*, 753–761.

[246] Hebels, D. G., Sveje, K. M., de Kok, M. C., van Herwijnen, M. H. et al., *N*-nitroso compound exposure-associated transcriptomic profiles are indicative of an increased risk for colorectal cancer. *Cancer Lett.* 2011, *309*, 1–10.

[247] Pignatelli, B., Chen, C.-S., Thuillier, P., Bartsch, H., in: Nicolai, H. V., Eisenbrand, G., Bozler, G. (Eds.), *The Significance of N-Nitrosation of Drugs*, Gustav Fischer Verlag, Stuttgart, New York 1990, pp. 123–141.

[248] Hebels, D. G., Sveje, K. M., de Kok, M. C., van Herwijnen, M. H. et al., Red meat intake-induced increases in fecal water genotoxicity correlate with pro-carcinogenic gene expression changes in the human colon. *Food Chem. Toxicol.* 2012, *50*, 95–103.

[249] Hughes, R., Cross, A. J., Pollock, J. R. A., Bingham, S., Dose-dependent effect of dietary meat on endogenous colonic *N*-nitrosation. *Carcinogenesis* 2001, *22*, 199–202.

[250] Cross, A. J., Pollock, J. R., Bingham, S. A., Haem, not protein or inorganic iron, is responsible for endogenous intestinal *N*-nitrosation arising from red meat. *Cancer Res.* 2003, *63*, 2358–2360.

[251] Bastide, N. M., Pierre, F. H. F., Corpet, D. E., Heme iron from meat and risk of colorectal cancer: a meta-analysis and a review of the mechanisms involved. *Cancer Prev. Res.* 2011, *4*, 177–184.

[252] Bonnett, R., Charalambides, A. A., Martin, R. A., Sales, K. D. et al., Reactions of nitrous acid and nitric oxide with porphyrins and haems. Nitrosylhaems as nitrosating agents. *J. Chem. Soc. Chem. Comm.* 1975, *21*, 884–885.

[253] Butler, A. R., Rhodes, P., Chemistry, analysis, and biological roles of *S*-nitrosothiols. *Anal. Biochem.* 1997, *249*, 1–9.

[254] Gay, L. J., Mitrou, P. N., Keen, J., Bowman, R. et al., Dietary, lifestyle and clinicopathological factors associated with *APC* mutations and promoter methylation in colorectal cancers from the EPIC-Norfolk study. *J. Pathol.* 2012, *228*, 405–415.

[255] Oostindjer, M., Alexander, J., Amdam, G. V., Andersen, G. et al., The role of red and processed meat in colorectal cancer development. *Meat Sci.* 2014, *97*, 583–596.

[256] Sandhu, M. S., White, I. R., McPherson, K., Systematic review of the prospective cohort studies on meat consumption and colorectal cancer risk: a meta-analytical approach. *Cancer Epidemiol. Biomarkers Prev.* 2001, *10*, 439–446.

[257] Cross, A. J., Freedman, N. D., Ren, J., Ward, M. H. et al., Meat consumption and risk of esophageal and gastric cancer in a large prospective study. *Am. J. Gastroenterol.* 2011, *106*, 432–442.

[258] Chan, D. S. M., Lau, R., Aune, D., Vieira, R. et al., Red and processed meat and colorectal cancer incidence: meta-analysis of prospective studies. *PLoS One* 2011, *6*, e20456.

[259] Sinha, R., Cross, A. J., Graubard, B. I., Leitzmann, M. F. et al., Meat intake and mortality: a prospective study of over half a million people. *Arch. Int. Med.* 2009, *169*, 562–571.

[260] Ward, M. H., Cantor, K. P., Riley, D., Merkle, S. et al., Nitrate in public water supplies and risk of bladder cancer. *Epidemiology* 2003, *14*, 183–190.

[261] Ward, M. H., Cerhan, J. R., Colt, J. S., Hartge, P., Risk of non-Hodgkin lymphoma and nitrate and nitrite from drinking water and diet. *Epidemiology* 2006, *17*, 375–382.

[262] Kilfoy, B. A., Zhang, Y., Park, Y., Holford, T. R. et al., Dietary nitrate and nitrite and the risk of thyroid cancer in the NIH-AARP Diet and Health Study. *Int. J. Cancer* 2011, *129*, 160–172.

[263] Cross, A. J., Ferrucci, L. M., Risch, A., Graubard, B. I. et al., A large prospective study of meat consumption and colorectal cancer risk: an investigation of potential mechanisms underlying this association. *Cancer Res.* 2010, *70*, 2406–2414.

[264] Dubrow, R., Darefsky, A. S., Park, Y., Mayne, S. T. et al., Dietary components related to *N*-nitroso compound formation: a prospective study of adult glioma. *Cancer Epidemiol. Biomarkers Prev.* 2010, *19*, 1709–1722.

[265] Aschebrook-Kilfoy, B., Ward, M. H., Gierach, G. L., Schatzkin, A. et al., Epithelial ovarian cancer and exposure to dietary nitrate and nitrite in the NIH-AARP Diet and Health Study. *Eur. J. Cancer Prev.* 2012, *21*, 65–72.

[266] Dellavalle, C. T., Daniel, C. R., Aschebrook-Kilfoy, B., Hollenbeck, A. R. et al., Dietary intake of nitrate and nitrite and risk of renal cell carcinoma in the NIH-AARP Diet and Health Study. *Br. J. Cancer* 2013, *108*, 205–212.

[267] Ferrucci, L. M., Sinha, R., Ward, M. H., Graubard, B. I. et al., Meat and components of meat and the risk of bladder cancer in the NIH-AARP Diet and Health Study. *Cancer* 2010, *116*, 4345–4353.

[268] Aschebrook-Kilfoy, B., Cross, A. J., Stolzenberg-Solomon, R. Z., Schatzkin, A. et al., Pancreatic cancer and exposure to dietary nitrate and nitrite in the NIH-AARP Diet and Health Study. *Am. J. Epidemiol.* 2011, *174*, 305–315.

[269] Daniel, C. R., Cross, A. J., Graubard, B. I., Park, Y. et al., Large prospective investigation of meat intake, related mutagens, and risk of renal cell carcinoma. *Am. J. Clin. Nutr.* 2012, *95*, 155–162.

[270] Huncharek, M., Kupelnick, B., Wheeler, L., Dietary cured meat and the risk of adult glioma: a meta-analysis of nine observational studies. *J. Environ. Pathol. Toxicol. Oncol.* 2003, *22*, 129–137.

[271] Michaud, D. S., Holick, C. N., Batchelor, T. T., Giovannucci, E. et al., Prospective study of meat intake and dietary nitrates, nitrites, and nitrosamines and risk of adult glioma. *Am. J. Clin. Nutr.* 2009, *90*, 570–577.

[272] Terry, M. B., Howe, G., Pogoda, J. M., Zhang, F. F. et al., An international case-control study of adult diet and brain tumor risk: a histology-specific analysis by food group. *Ann. Epidemiol.* 2009, *19*, 161–171.

[273] Loh, Y. H., Jakszyn, P., Luben, R. N., Mulligan, A. A. et al., N-nitroso compounds and cancer incidence: the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk Study. *Am. J. Clin. Nutr.* 2011, *93*, 1053–1061.

[274] Jakszyn, P., Bingham, S., Pera, G., Agudo, A. et al., Endogenous versus exogenous exposure to N-nitroso compounds and gastric cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC-EURGAST) study. *Carcinogenesis* 2006, *27*, 1497–1501.

Addendum

[2]Department of Molecular and Cellular Sports Medicine, German Sport University Cologne, Cologne, Germany
[3]Chair of General Food Technology, Center of Life and Food Sciences Weihenstephan, Technical University of Munich, Freising-Weihenstephan, Germany
[4]Institute of Pharmacy and Biochemistry, University of Mainz, Mainz, Germany
[5]Chemical and Veterinary Analytical Institute Münsterland-Emscher-Lippe, Münster, Germany
[6]German Institute of Food Technologies, Quakenbrück, Germany
[7]Institute of Food Chemistry, Westfälische Wilhelms-Universität Münster, Münster, Germany
[8]German Institute of Human Nutrition, Potsdam-Rehbrücke, Germany
[9]Department of Food Biotechnology and Food Process Engineering, Technical University of Berlin, Berlin, Germany
[10]Department of Health Risk Analysis and Toxicology, Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, The Netherlands
[11]Department of Safety and Quality of Fruit and Vegetables, Federal Research Institute of Nutrition and Food, Max Rubner-Institut, Karlsruhe, Germany
[12]Federal Institute for Risk Assessment, Berlin, Germany
[13]Department of Food Chemistry and Toxicology, University of Vienna, Vienna, Austria
[14]Division of Toxicology, Wageningen University, Wageningen, The Netherlands
[15]Nestec S.A., Vevey, Switzerland
[16]Paul-Ehrlich-Institut, Langen, Germany
[17]Chair of Technical Microbiology, Center of Life and Food Sciences Weihenstephan, Technical University of Munich, Freising-Weihenstephan, Germany
[18]Institute for Food Toxicology and Analytical Chemistry, University of Veterinary Medicine Hannover, Hannover, Germany

