

Technical University of Denmark



Dietary exposure to volatile and non-volatile N-nitrosamines from processed meat products in Denmark

Herrmann, Susan Strange; Duedahl-Olesen, Lene; Christensen, Tue; Olesen, Pelle Thonning; Granby, Kit

Published in:
Food and Chemical Toxicology

Link to article, DOI:
[10.1016/j.fct.2015.03.008](https://doi.org/10.1016/j.fct.2015.03.008)

Publication date:
2015

Document Version
Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

Citation (APA):
Herrmann, S. S., Duedahl-Olesen, L., Christensen, T., Olesen, P. T., & Granby, K. (2015). Dietary exposure to volatile and non-volatile N-nitrosamines from processed meat products in Denmark. *Food and Chemical Toxicology*, 80, 137-143. DOI: 10.1016/j.fct.2015.03.008

DTU Library

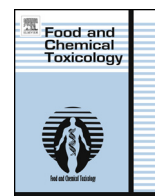
Technical Information Center of Denmark

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.



Dietary exposure to volatile and non-volatile *N*-nitrosamines from processed meat products in Denmark

S.S. Herrmann*, L. Duedahl-Olesen, T. Christensen, P.T. Olesen, K. Granby

National Food Institute, Technical University of Denmark, Mørkhøj Bygade 19, Søborg DK-2860, Denmark

ARTICLE INFO

Article history:

Received 28 October 2014

Accepted 11 March 2015

Available online 16 March 2015

Keywords:

Nitrite curing
N-nitroso compounds
 Dietary exposure
 Risk assessment

ABSTRACT

Recent epidemiological studies show a positive association between cancer incidence and high intake of processed meat. *N*-nitrosamines (NAs) in these products have been suggested as one potential causative factor. Most volatile NAs (VNAs) are classified as probable human carcinogens, whereas the carcinogenicity for the majority of the non-volatile NA (NVNA) remains to be elucidated. Danish adults (15–75 years) and children (4–6 years) consume 20 g and 16 g of processed meat per day (95th percentile), respectively. The consumption is primarily accounted for by sausages, salami, pork flank (spiced and boiled) and ham. This consumption results in an exposure to NVNA of 33 and 90 ng kg bw⁻¹ day⁻¹ for adults and children, respectively. The exposure to VNA is significantly lower amounting to 0.34 and 1.1 ng kg bw⁻¹ day⁻¹ for adults and children, respectively. Based on a BMDL10 of 29 µg kg bw⁻¹ day⁻¹ a MOE value ≥17,000 was derived for the exposure to NA known to be carcinogenic (VNA including NSAR), indicating an exposure of low concern. The exposure to the NVNA is substantially higher and if found to be of toxicological significance the exposure may be of concern.

© 2015 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Several epidemiologic studies show associations between consumption of red and processed meat and increased risk of, e.g. colorectal cancer (Santarelli et al., 2008), stomach cancer (Larsson et al., 2006), pancreatic cancer (Larsson and Wolk, 2012) as well as increased risk of cardiovascular diseases and other causes of death (Rohrman et al., 2013). The association was stronger for high consumption of processed meat than for high consumption of red meat in several of these studies. In 2007 the scientifically based evidence led the World Cancer Research Fund to recommend that consumption of processed meat should be avoided whereas the Danish food authorities recommend limiting the consumption of processed meat (www.foedevarestyrelsen.dk). Rohrman et al. (2013) estimated that consumption of more than 20 g of processed meat per day increased the mortality rate (Rohrman et al., 2013).

Processed meat often is signified by the use of nitrite (E 249–E 250) or nitrate (E 251–E 252) for preservation, salting and, for some products, also smoking. Meat products preserved with nitrite and/or nitrate are associated with the occurrence of *N*-nitrosamines (NAs), of which many are genotoxic and classified as probable human carcinogens (IARC, 1978). The so called volatile NA (VNA), which include,

e.g. *N*-nitrosodimethylamine (NDMA), *N*-nitrosopyrrolidine (NPYR), *N*-nitrosopiperidine (NPIP) and *N*-nitrosodiethylamine (NDEA), occurs generally at low levels <5 µg kg⁻¹ but levels up to 20 µg kg⁻¹ has been reported (Hill et al., 1988; Massey et al., 1991). NDEA has been evaluated as the most potent carcinogen among the known meat related VNAs (Peto et al., 1984). The non-volatile NAs (NVNAs), which include the *N*-nitrosamino acids, e.g. *N*-nitrosohydroxyproline (NHPRO), *N*-nitrosoproline (NPRO), *N*-nitrososarcosine (NSAR), *N*-nitrosothiazolidine-4-carboxylic acid (NTCA), *N*-nitroso-2-methylthiazolidine-4-carboxylic acid (NMTCA), generally occur at significantly higher levels than the VNAs, i.e. up to several thousand microgram per kilo (Herrmann et al., 2014a; Massey et al., 1991; Tricker and Kubacki, 1992). With the exception of NHPRO and NPRO the carcinogenicity of the NVNAs are poorly elucidated (Tricker et al., 1991). For NTCA and NMTCA a literature study only revealed three *in vitro* genotoxicity studies of limited scope (Lin and Gruenwedel, 1990; Negishi et al., 1991; Umamo et al., 1984) for NTCA and no studies for NMTCA. Thus, the toxicological significance of several of the NVNAs cannot be evaluated because of insufficient data.

Estimations of the dietary exposure to NAs are available in the literature though only for the VNA, NDMA, NPYR and in a few cases NPIP. Studies on the dietary exposure to NDMA published from 1978 to 1990 have been reviewed by Tricker et al., (1991) (Table 1). Since then others have published results on dietary exposure to NDMA, NPYR and NPIP (Table 1). The estimated exposures to VNA from all foods range from 80 ng day⁻¹ (NDMA only) for the Finish population (1990), to 900 ng day⁻¹ (NDMA, NPYR, NPIP) for the German

* Corresponding author. National Food Institute, Technical University of Denmark, Mørkhøj Bygade 19, Søborg, DK-2860 Denmark.
 E-mail address: sher@food.dtu.dk (S.S. Herrmann).

Table 1
Dietary exposure levels for *N*-nitrosamines estimated by others. The exposure values are for adults unless otherwise stated.

Compound	Exposure level	Food source (population)	Reference
NDMA	0.1 $\mu\text{g day}^{-1}$	Food and beverages (the Netherlands)	Keszei et al., 2013
NDMA	0.03 $\mu\text{g day}^{-1a}$ 0.04 $\mu\text{g day}^{-1}$ (1 year old) ^a	Fish/vegetables meals (the Netherlands)	Zeilmaker et al., 2010
NDMA	0.08 $\mu\text{g day}^{-1}$ (average bw 60 kg)	Food (Finland)	Penttila et al., 1990
Sum of NDMA, NPYR and NPIP	0.9 $\mu\text{g day}^{-1}$	Food (Germany 1980)	Tricker et al., 1991
Sum of NDMA, NPYR and NPIP	0.3 $\mu\text{g day}^{-1}$	Food (Germany 1990)	Tricker et al., 1991
NDMA	0.09 $\mu\text{g day}^{-1}$	Meat products (Germany in 1990)	Tricker et al., 1991
NPYR	0.0016 $\mu\text{g day}^{-1}$	Meat products (Germany in 1990)	Tricker et al., 1991
NPIP	0.0055 $\mu\text{g day}^{-1}$	Meat products (Germany in 1990)	Tricker et al., 1991
VNA	0.3–1 $\mu\text{g day}^{-1}$	Food (with cured meats and beer as major sources) (Western countries in average)	Tricker and Preussmann, 1991
NVNA	~10–100 $\mu\text{g day}^{-1}$	Food (Western countries in average)	Tricker and Preussmann, 1991

^a The exposure level was presented in $\text{ng kg bw}^{-1} \text{day}^{-1}$ and in order to have all the values presented as $\mu\text{g day}^{-1}$ the values for a 1 year old and adults have been multiplied with 9.5 kg or 75 kg, respectively.

(1980) and 1000 ng day^{-1} (NDMA, NPYR) for the Swiss population (1992) (Tricker et al., 1991) (Table 1). According to the estimates performed for the German population in 1980 and in 1990 the VNA exposure level was reduced to one third during this period. This was primarily due to a decrease in the levels of NDMA in beer but also due to a decrease in the content in meat products. Because of lower levels of NDMA in beer, meat products became the primary dietary source of NDMA. Meat products were the second largest source of dietary NPYR and NPIP only surpassed by spices (Tricker et al., 1991). The exposures to NDMA, NPYR and NPIP from meat products were estimated to be 90, 1.6 and 5.5 ng day^{-1} , respectively (Germany 1990). Another important source of VNA is fish products, being the major source of NDMA exposure in some countries (Tricker et al., 1991). Recently the exposure of the Dutch population to NDMA from food and beverages was estimated to range from 2 to 2701 ng day^{-1} with a mean of 109 ng day^{-1} (Keszei et al., 2013) (Table 1). Thus, meat products, which include processed meat, are in most cases the main source of VNA. So far insufficient data exist on the occurrence of NVNA in order to perform an estimation of the dietary exposure. It was however suggested by Tricker and Preussmann (1991) to be in the order of 10–100 $\mu\text{g day}^{-1}$ (Tricker and Preussmann, 1991), thus significantly higher than the estimated dietary exposure to VNA. Since the 1970s the levels of NA in processed meat have decreased, because of the introduction of regulation and restriction on the use of nitrite and nitrate and because it has become more common to also add antioxidants which limit the NA formation. However the dietary exposure to NA from processed meat may still pose a health risk due to the increased consumption of processed meat in several European countries including Denmark (Linseisen et al., 2002).

The Danish population's exposure to VNA and NVNA from processed meat products has so far not been assessed. Primarily due to concern for the formation of NA the Danish authorities have found it necessary to maintain national provisions imposing stricter

limits on the use of nitrites than the EU. These national provisions allow the addition of maximum 60 mg kg^{-1} to most meat products intended for the Danish market (Commission decision, L 247/55 of 25 May 2010). The common EU legislation (Directive 2006/52/EC) allow the addition of 150 mg kg^{-1} meat. This exception expires in May 2015 and in order to evaluate what the consequence will be for the Danish population if more nitrite may be added during meat processing, an evaluation of the exposure levels, as it is now, is needed. Recently we performed a survey on the occurrence of VNA as well as NVNA in processed meat products on the Danish market (Herrmann et al., 2014a). Survey data from the 70 analyzed samples can provide a preliminary estimation of the exposure to VNA and NVNA from processed meat products for the Danish populations.

The aim of the present study is therefore to estimate the exposure to VNA and NVNA from processed meat products for the Danish population based on results of the recently performed Danish survey and data on consumption of processed meat products by the Danish population as well as for the high consumers, the 95th percentile. Risk characterization is performed by estimating the margin of exposure (MOE) to NA, based on the ratio between a benchmark dose level (BMDL₁₀) and the estimated dietary exposures.

2. Materials and methods

2.1. Contents of NAs in processed meat products

Results from a recently performed survey on the occurrence of NAs in 70 samples of processed meat products available on the Danish market were applied for the exposure assessment. Details on this study are described elsewhere (Herrmann et al., 2014a). The NA contents of the samples were determined using a recently developed method allowing for the quantification of eight VNAs and five NVNAs (Herrmann et al., 2014b). In brief the homogenized samples (2.5 g) were extracted with acidified acetonitrile (7.5 ml with 1% formic acid). After centrifugation the clear supernatant was frozen, defrosted and centrifuged again. An aliquot was concentrated by a factor of five under a gentle stream of nitrogen. An aliquot was mixed 1:1 with Milli-Q water, filtered and analyzed by LC (APCI/ESI)-MS/MS. The chromatographic separation was performed on an Agilent 1200 Series HPLC (Agilent Technologies, Santa Clara, CA, USA) with a Poroshell PhenylHexyl 150 \times 2.1 mm, 3 μm column (Agilent Technologies) using water and methanol both with 0.1% formic acid as mobile phase. The MS/MS detection was performed on an Agilent 6460 Series Triple Quadrupole (Agilent Technologies) equipped with either an APCI or a Jet Stream ESI source. The quantitative and qualitative analyses were performed by external calibration and comparing retention times and quantifier ion/qualifier ion ratios obtained by analyzing NA standard solutions and spiked QC samples and comparing with the samples. The LOQs obtained with the described method were generally <1 mg kg^{-1} , though with some exceptions for specific NA/meat product combinations. The validation results are presented in detail in Herrmann et al., 2014b.

The results of the survey are presented as the mean content in Table 2. Both the mean of all positive findings as well as the mean of all samples analyzed are presented. The latter mean values are the values applied for the exposure assessment. The non-detects were in this case set to zero.

2.2. Exposure assessment

Intakes of NA via processed meat products were estimated using two representative groups, i.e. Danish children 4–6 years of age and Danish Adults 15–75 years of age. The exposure level for the 6–14 year old children was not calculated because the consumption of processed meat is comparable to the consumption by the 4–6 year olds (Table 3) and the latter group will therefore be the most exposed group of the two.

Consumption data from the Danish National Survey of Diet and Physical Activity (DANSDA) (Pedersen et al., 2010) was used for estimation of the NA exposure for both groups. In this survey consumption of food and drink was recorded for seven consecutive days from a representative sample of 2700 Danes aged 4–75. The consumption data for each individual participant in the survey were available for the dietary estimation performed in the present work. The types of processed meat products traditionally preserved with nitrite included in the survey were: ham (specific recording of either boiled and smoked ham, boiled and canned ham or boiled and sliced ham), bacon, salami, sausages, medister sausage (raw sausage, Danish speciality), pork flank (spiced and boiled), meat sausage (pork based luncheon meat), smoked pork fillet, kassler (smoked, boiled pork saddle), salted meat (pork, luncheon meat), chicken breast (boiled, luncheon meat). More details on how the survey and handling of the consumption data were performed are available in Pedersen et al., 2010.

Table 2

Mean levels of VNA (uncolored columns) and NVNA (gray columns) ($\mu\text{g kg}^{-1}$) in processed meat products on the Danish market. The mean of all quantifiable contents and mean of all contents including non-detects each compound.

Compound name	Abbreviation	Mean content (positive findings) ^a	Mean content (all findings) ^a	Samples with content (%)	Highest content ($\mu\text{g kg}^{-1}$)
N-nitrosodimethylamine	NDMA	1.3	0.7	50	4.0
N-nitrosomorpholine	NMOR	0.1	0.01	7	0.5
N-nitrosomethylethylamine	NMEA	0.3	0.04	13	0.5
N-nitrosopyrrolidine	NPYR	2	0.8	47	13
N-nitrosodiethylamine	NDEA	0.3	0.04	16	0.3
N-nitrosopiperidine	NPIP	0.1	0.02	13	0.2
N-nitrososarcosine	NSAR	20	1.5	7	82
N-nitrosoproline	NPRO	4.3	2.3	53	30
N-nitrosomethylaniline	NMA	0.47	0.1	21	0.9
N-nitroso-thiazolidine-4-carboxylic acid	NTCA	120	118	99	2034
N-nitroso-2-methyl-thiazolidine-4-carboxylic acid	NMTCA	8.1	7.3	90	39

^a The means are based on all positive findings including those below LOQ if the retention times and ion ratios matched those of the standard solutions or quality control samples. Non-detects were not included when calculating the means for the individual product groups nor when calculating the mean of “positive results, all products”. When calculating the means of “all results, all products” non-detects were set to zero.

2.3. Risk characterization

In order to evaluate the significance of an estimated dietary exposure to genotoxic compounds the exposure basically compared with the dose leading to a specified incidence of tumor formation in experimental animals. The larger the margins between the effect dose level and the actual exposure level (margins of exposure, MOE) the lower is the concern. An internationally recognized toxicological reference point is the Benchmark Dose Lower confidence Limit (BMDL), which represents the exposure level where an increase in the incidences of the effect (at 10% in case of animal experiments) is smaller than the specified Benchmark Response with a confidence of 95% (Zeilmaker et al., 2010). The BMDL₁₀ may be derived from dose–response data from several long-term carcinogenicity studies instead of one extensive study. For genotoxic compounds EFSA has expressed that if the BMDL₁₀ is 10,000 times higher than the exposure (i.e. MOE of 10,000), the exposure is of low concern (EFSA, European Food Safety Authority, 2012).

3. Results and discussion

3.1. Processed meat consumption and occurrence data

The 95th percentile of the mean consumption of processed meat consumption in Denmark derived from DANSDA (Pedersen et al., 2010) is summarized in Table 3. The amounts of processed meat consumed by the three different age groups are all in the range of 16–20 g per person per day. If the body weights (bw) of the three

Table 3

Consumption of processed meat products (gram day⁻¹ person⁻¹ for Danish children and adults as mean and at the 95th percentile, derived from the Danish National Survey of Diet and Physical Activity (DANSDA) (Dietary habits in Denmark 2003–2008) (Pedersen et al., 2010).

g/day (Mean)	4–5 Years (bw: 18 kg)	6–14 Years (bw: 30 kg)	15–75 Years (bw: 72 kg)
Sausages	2.0	1.6	1.3
Salami	1.2	1.3	1.1
Pork flank, spiced, boiled (luncheon meat)	0.9	0.8	1.0
Medister sausage (Danish special product)	0.6	0.4	0.4
Ham	0.9	1.1	1.6
Kassler (luncheon meat)	0.6	0.5	0.8
Salted meat (luncheon meat)	0.3	0.3	0.5
Meat sausage (luncheon meat, pork based)	0.3	0.3	0.4
Chicken and turkey fillet (luncheon meat)	0.1	0.2	0.1
Bacon	0.1	0.2	0.4
Fillet, pork, smoked	0.2	0.2	0.3
Total	6.9	6.5	7.5
Total 95th percentile	16.2	16.8	20.2
Largest daily consumption	22.0	56.6	53.0

BW: body weight.

groups are taken into account, the 4–6 year olds (bw 18 kg) and the 6–14 year olds (bw 30 kg) consume about three and two times more processed meat per day than the 15–75 year olds (bw 72 kg), respectively. Thus, the 4–6 year old children will, because of their lower bodyweight, have the highest exposure ($\text{ng kg bw}^{-1} \text{ day}^{-1}$). The types of processed meat consumed by the three groups are similar (Table 3). Sausages contribute most to the total consumption (25–30%) and salami accounts for the second largest fraction for both groups of children (13–20%). Pork flank (4–5 years) or ham (6–14 and 15–75 years) is the third most consumed product. Thus the high consumers, defined by the 95th percentile, of Danish population, consume on average 1.5 times the amount (20 g) reported to affect the mortality (Rohrmann et al., 2013).

According to data from the European Prospective Investigation into Cancer and Nutrition (EPIC) the total consumption of processed meat product by the Danish population is similar to the consumption by the French adults, though only about half of that reported for the German and Norwegian adults. Sausages also account for the major part of the processed meats consumed by the French, German and Norwegian adults (Linseisen et al., 2002). A higher consumption of sausages accounts for the major part of the higher total consumption by the German and Norwegian adults. According to the survey summarized in Table 2 the NVNAs are detected in nearly all samples taken from the Danish market. The mean levels of the individual NVNAs were $\leq 118 \mu\text{g kg}^{-1}$, highest for NTCA. NTCA and NMTCA are found at concentration levels up to $4030 \mu\text{g kg}^{-1}$ and $39 \mu\text{g kg}^{-1}$, respectively.

VNAs are also detected in several samples though at considerably lower levels. The mean levels of the individual VNAs in samples from the Danish market are $\leq 0.8 \mu\text{g kg}^{-1}$. Of the targeted NA only N-nitrosodibutylamine is not found. NDBzA and NDPA were detected in a few samples and NHPRO were detected in about 40% of the samples. However, the contents of these three NAs could not be quantified with enough certainty using the developed method and contents of these have therefore not been included in the exposure calculation.

3.2. Exposure assessment

The estimated mean exposure and the 95th percentile for the individual NAs and the sum of NVNAs and VNAs are presented in Fig. 1A/1C and 1B/1D, respectively.

The 4 to 5 year old children have the highest consumption of processed meat ($\text{g kg bw}^{-1} \text{ day}^{-1}$) and therefore also the highest exposure to NVNAs and VNAs, i.e. $90 \text{ ng kg bw}^{-1} \text{ day}^{-1}$ (mean $37 \text{ ng kg bw}^{-1} \text{ day}^{-1}$) and $1.1 \text{ ng kg bw}^{-1} \text{ day}^{-1}$ (mean $0.45 \text{ ng kg bw}^{-1} \text{ day}^{-1}$), respectively (Fig. 1A and 1B). The total exposure to NVNAs and VNAs

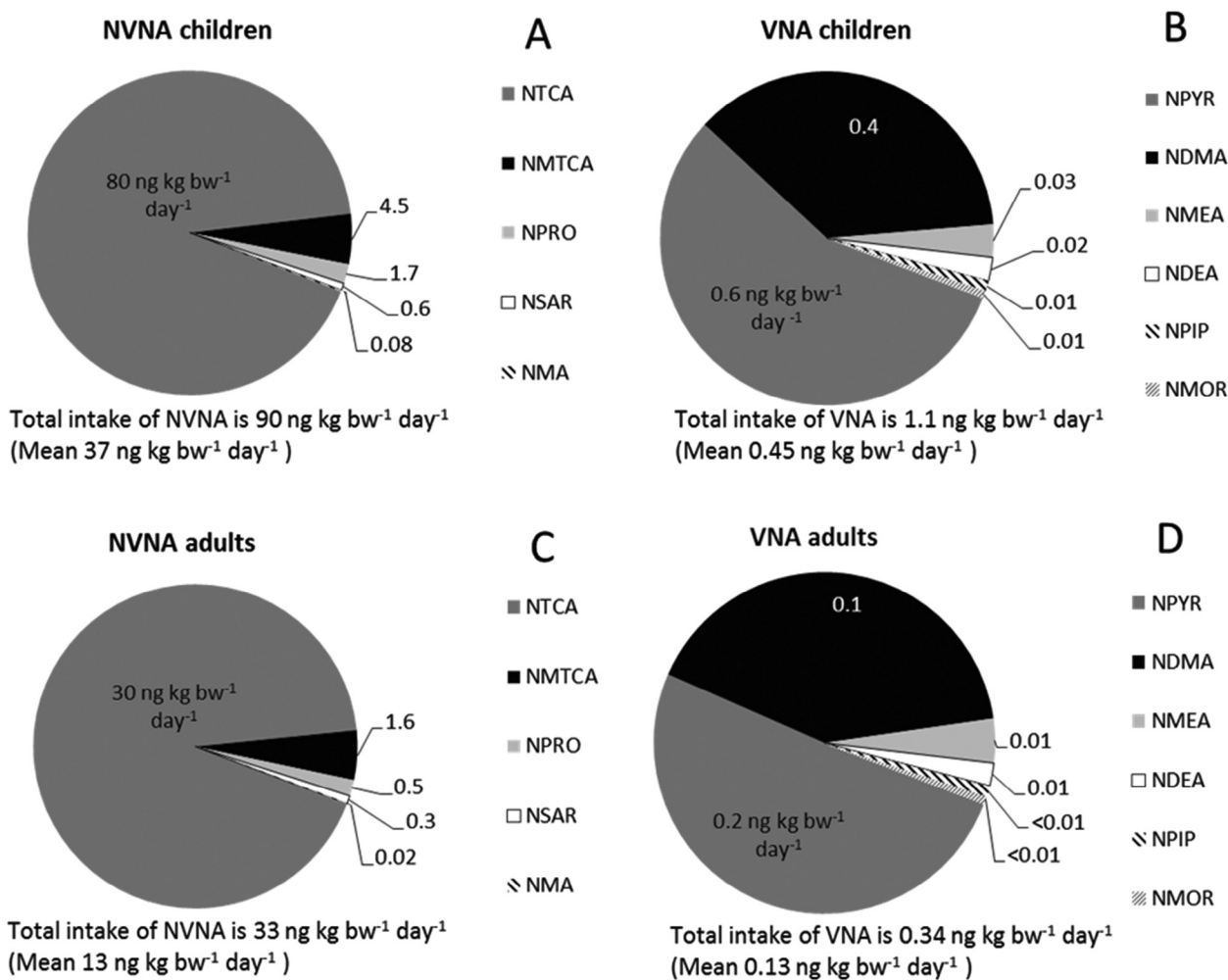


Fig. 1. Exposure levels (ng kg bw⁻¹ day⁻¹) (95th percentile) to NVNA (A and C) and VNA (B and D) by consumption of processed meat products for children (4–5 years of age) (A and B) and adults (15–75 years of age) (C and D).

for adults was 33 and 0.34 ng kg bw⁻¹ day⁻¹ (mean 13 and 0.13 ng kg bw⁻¹ day⁻¹), respectively (Fig. 1C and 1D). NTCA accounted for about 90% of the total exposure to NVNAs for both children and adults. NMTCA and NPRO accounted for approximately 5 and 2% of the NVNA exposure, respectively. For the VNAs, NPYR and NDMA accounted for about 50% and 40% of the total exposure to VNAs for both children and adults, respectively (Fig. 1B and 1D). The classical VNAs (NDMA, NPYR, NPIP, NDEA) accounted for >90% of the exposure to VNAs. The exposure levels estimated in the present work for the VNA are in the same order of magnitude as the exposure levels reported by others (Table 1) (Keszei et al., 2013; Tricker et al., 1991). The exposure levels estimated for the NVNA though seems to be lower than the intake suggested by Tricker and Preussmann (1991). Since other NVNAs besides those included in the present study have been identified in processed meat (Janowski et al., 1978; Sen et al., 1993; Tricker and Kubacki, 1992), the estimated exposure might thus be underestimated.

Ham and salami accounted for about 75% of the exposure to NVNA (Fig. 2A and 2C). Ham, salami and sausages accounted for about 70 and 80% of the exposure to VNA for adults and children, respectively (Fig. 2B and 2D). Thus, the present results indicate that ham, salami and sausages are the primary meat source of NA for the Danish population. In Germany a higher intake of NA is expected, partly due to the greater consumption of processed meat (Linseisen et al., 2002) and partly due to the less restrictive EU

regulation on the use of nitrite for meat preservation, allowing for more nitrite to be added than the Danish provisions (Commission decision, L 247/55 of 25 May 2010). If more nitrite is added during processing the levels of NA generally increase (Gry et al., 1983; Herrmann et al., 2015). The primary meat source of NA is most likely sausages for the German and Norwegian populations since they consume 4–7 times more sausages than the Danish population (Linseisen et al., 2002).

3.3. Risk assessment

The NAs are relatively stable compounds but are activated metabolically, via hydroxylation catalyzed by enzymes of the cytochrome P450 family, and thereby they become carcinogenic. NDMA and NDEA are the most studied NA with regard to toxicity. Both NDMA and NDEA are carcinogenic in all of the animals they have been tested. The target organs are liver, respiratory tract and kidney (IARC, 1978). Because the NA needs to be metabolically activated to become carcinogenic the target organs are those with activity of the P450 enzyme with affinity for the relevant NA. E.g. NDMA is readily metabolized in the rat liver, less in rat kidney and lung, and consequently liver tumors are the primary endpoint in rats (Shank, 1975). Total liver tumors were found to be the most sensitive endpoint for rats exposed to NDMA (Zeilmaker et al., 2010). NPYR is non-carcinogenic in the rat esophagus whereas NPIP is a potent rat esophagus

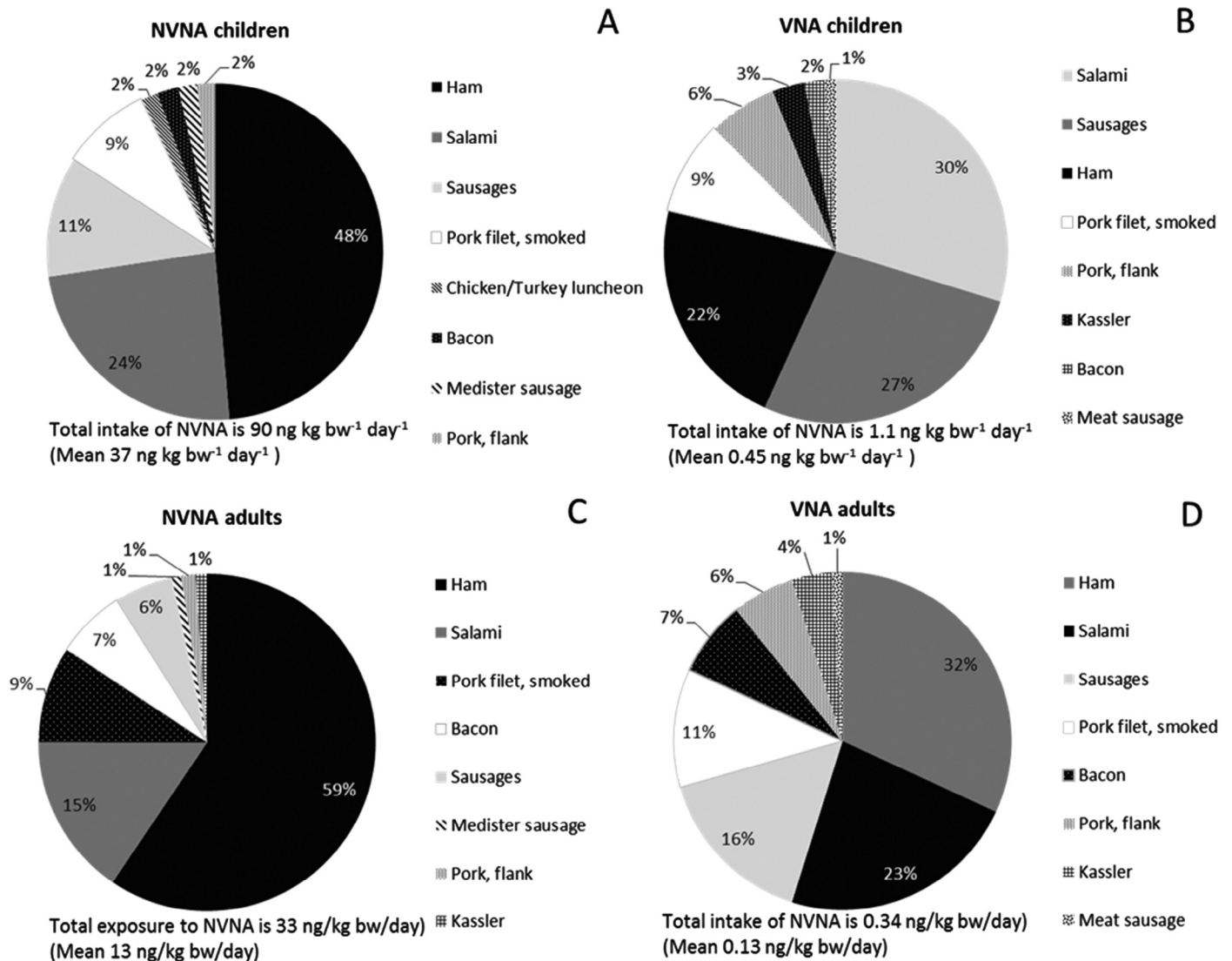


Fig. 2. Processed meat products contributing to the exposure to NVNA (A and C) and VNA (B and D) for children (4–5 years of age) (A and B) and adults (15–75 years of age) (C and D).

carcinogen. The activities of several P450 enzymes, including the P450A subfamily, are in humans associated with polymorphism and are inducible by several xenobiotics (Su and Ding, 2004). Thus the degree to which NAs are metabolically activated may vary between individuals and result in variation in susceptibility to NA exposure. It is assumed that the more polar the NAs are rapidly excreted and therefore less likely to be metabolically activated. This is part of the reason why the hydroxylated NAs are less potent carcinogens than their un-substituted NAs (Lijinsky 1987, 301–356).

A large number of studies on the carcinogenicity of the NA are available. The majority of these are smaller studies performed during the 1960s and 1970s. In general these studies show that long-term exposure of rats to NDMA or NDEA at levels of around 4 mg kg bw⁻¹ day⁻¹ leads to the development of tumors in up to 100% of the test animals (IARC, 1978). In general studies of sufficient quality and extent to allow for the estimation of Bench Mark Dose (BMD) are limited. However, a comprehensive chronic administration study performed with 16 different concentrations of NDMA in water given to rats was performed by Peto et al. (1984). This study has been used by several researchers to define a BMDL value (Table 4), e.g. by Dybing et al. (2008) and Zeilmaker et al. (2010) to estimate a BMDL₁₀

value for NDMA. Zeilmaker et al. 2010 derived a BMDL₁₀ of 29 µg kg bw⁻¹ day⁻¹ for NDMA chronic exposure when using total liver tumors as the most sensitive marker. This BMDL₁₀ value is in good agreement with the BMDL₁₀ of 27 µg kg bw⁻¹ day⁻¹ applied by the Scientific Committee on Consumer Safety (Scientific Committee on Consumer Safety, 2012). Dybing et al. (2008) on the other hand derived a BMDL₁₀ value of 62 µg kg bw⁻¹ day⁻¹ when using incidence of liver cell tumors as marker.

The different VNAs vary in their carcinogenic potency. NDMA, NPYR and NPIP all affected the same endpoint, i.e. total liver tumors but with descending potency, with NDMA being the most potent of the three (Peto et al., 1984). In the present study the BMDL₁₀ of 29 µg kg bw⁻¹ day⁻¹ was conservatively chosen for the combined risk assessment for the total of VNA. Except for NDEA the VNAs are less potent carcinogens than NDMA. NSAR is a carcinogenic NVNA (IARC, 1978), though a much weaker one than NDMA. For a conservative approach we therefore found it relevant to include NSAR in the summed exposure to carcinogenic NA and thereby include it in the risk assessment.

With a VNA exposure level of 1.1 ng kg bw⁻¹ day⁻¹ and NSAR of 0.6 ng kg bw⁻¹ day⁻¹ (total carcinogenic NA of 1.7 ng kg bw⁻¹ day⁻¹)

Table 4
Bench mark dose levels (BMDL) applied previously in the literature for carcinogenic risk assessment.

Compound	BMDL ₁₀	Based on what	Reference
NDMA	62 µg kg bw ⁻¹ day ⁻¹	Incidence of liver cell tumors in male Colworth Wistar rats	BMDL value set by Dybing et al. 2008 based on toxicological study by Peto et al. 1984
NDMA	29 µg kg bw ⁻¹ day ⁻¹	Total liver tumors in male Colworth Wistar rats	BMDL value set by Zeilmaker et al. 2010 based on toxicological study by Peto et al. 1984
NDMA	27 µg kg bw ⁻¹ day ⁻¹	Fatal liver neoplasm (rat)	Scientific Committee on Consumer Safety, 2012
NPYR	160 µg kg bw ⁻¹ day ⁻¹	Total liver tumor (rat)	Scientific Committee on Consumer Safety, 2012
NDEA	18 µg kg bw ⁻¹ day ⁻¹	Total liver tumors (rat)	Scientific Committee on Consumer Safety, 2012

The T25 approach is defined as the chronic dose rate (usually expressed in units of mg per kg bodyweight per day) which will give tumors at a specific tissue site in 25% of the animals after correction for spontaneous incidence and within the standard lifetime of the species (Dybing et al., 2008).

for children and a BMDL₁₀ of 29,000 ng kg bw⁻¹ day⁻¹, the MOE is 17,000 for NA originating from processed meat and is thus higher than 10,000. By applying the same approach for adults the MOE is found to be 45,000 for NA originating from processed meat.

Hence the present risk assessment of the exposure to VNA from processed meat products is that it may be considered of low concern, a conclusion which is in accordance with the evaluation by the European Food Safety Authority (EFSA). However, the VNAs are genotoxic compounds and a “no effect level” may therefore not exist. Even small exposure levels may still have a genotoxic effect (Dybing et al., 2008). Further, it should also be cautioned that the results from this study are based on fairly limited number of results on the occurrence of NA in processed meat products.

Assuming that only the VNAs are of toxicological relevance, there may still be reason to be concerned about the occurrence of NA in processed meats, even though the present study indicates that the NA exposure from processed meat on the Danish market is of low concern based on the estimated MOE. Firstly, the population is also exposed to NA from sources other than processed meat. Exposure data from sources other than processed meat are needed in order to make a complete risk assessment for the population. Secondly, as mentioned earlier, the carcinogenic potential of the majority of the NVNAs is unknown or only very limited information is available. These NVNAs can occur in much higher concentrations than the VNAs and in order to fully assess the risk of NA exposure from processed meat products further toxicological studies are needed on NVNAs. Thirdly, other unidentified carcinogenic NAs might be produced when the conditions allow for the formation of the known NA.

4. Conclusion

The Danish population consume as a 95th percentile 16–20 g of processed meat per day primarily consisting of sausages, salami, pork flank (spiced and boiled) and ham. The exposure to VNA by the consumption of processed meat was found to be low (0.34–1.1 ng kg bw⁻¹ day⁻¹), whereas the exposure to NVNA was considerably higher (33–90 ng kg bw⁻¹ day⁻¹). Adults (15–75 year old) and children (4–6 year old) consume almost the same amount of processed meat per day, resulting in a higher exposure for children because of their lower body weight. The calculated MOE (≥17,000) for the VNA exposure indicates that this is of low concern. In order to assess the significantly higher exposure to NVNAs the carcinogenic potential of these NAs needs to be elucidated.

Conflict of interest

The authors declare that there are no conflicts of interest.

Transparency document

The [Transparency document](#) associated with this article can be found in the online version.

Acknowledgement

This work was supported by a Danish research grant from the Ministry of Foods, Agriculture and Fisheries of Denmark, project Nitrosamines in meat products no. 3304-NIFA-11-0556.

References

- Dybing, E., O'Brien, J., Renwick, A.G., Sanner, T., 2008. Risk assessment of dietary exposures to compounds that are genotoxic and carcinogenic – An overview. *Toxicol. Lett.* 180, 110–117. <http://dx.doi.org/globalproxy.cvt.dk/10.1016/j.toxlet.2008.05.007>.
- EFSA, European Food Safety Authority, 2012. Statement on the applicability of the Margin of Exposure approach for the safety assessment of impurities which are both genotoxic and carcinogenic in substances added to food/feed. EFSA Scientific Committee.
- Gry, J., Rasmussen, N.J.D., Jensen, W.K., Brandt, I.G., Fabeck, B., 1983. Investigations on effects of nitrite in commercially prepared Danish cured meat products. The Federation of Danish Pig Producers and Slaughterhouses & The National Food Agency of Denmark. ISBN 8750344595, 9788750344599.
- Herrmann, S.S., Duedahl-Olesen, L., Granby, K., 2014a. Occurrence of volatile and non-volatile N-nitrosamines in processed meat products and the role of heat treatment. *Food Control* 48, 163–169.
- Herrmann, S.S., Duedahl-Olesen, L., Granby, K., 2014b. Simultaneous determination of volatile and non-volatile nitrosamines in processed meat products by liquid chromatography tandem mass spectrometry using atmospheric pressure chemical ionisation and electrospray ionisation. *J. Chromatogr. A* 1330, 20–29. <http://dx.doi.org/globalproxy.cvt.dk/10.1016/j.chroma.2014.01.009>.
- Herrmann, S.S., Granby, K., Duedahl-Olesen, L., 2015. Formation and mitigation of N-nitrosamines in nitrite preserved cooked sausages. *Food Chem.* 174, 516–526.
- Hill, M.J., Massey, R.C., Shuker, D.E.G., Leach, S., Tricker, A.R., Preussmann, R., et al., 1988. Nitrosamines. *Toxicology and Microbiology*. p. 169. ISBN 0-89573-605-0.
- IARC, 1978. IARC monographs on the evaluation of carcinogenic risks to humans. Some N-nitroso compounds. IARC Monogr. Eval. Carcinog. Risks Hum. 17, 1–349.
- Janzowski, C., Eisenbrand, G., Preussmann, R., 1978. Occurrence of N-nitrosamino acids in cured meat products and their effect on formation of N-nitrosamines during heating. *Food Cosmet. Toxicol.* 16, 343–348. [http://dx.doi.org/globalproxy.cvt.dk/10.1016/S0015-6264\(78\)80005-4](http://dx.doi.org/globalproxy.cvt.dk/10.1016/S0015-6264(78)80005-4).
- Keszei, A.P., Goldbohm, R.A., Schouten, L.J., Jakszyn, P., van den Brandt, P.A., 2013. Dietary N-nitroso compounds, endogenous nitrosation, and the risk of esophageal and gastric cancer subtypes in the Netherlands Cohort Study. *Am. J. Clin. Nutr.* 97, 135–146.
- Larsson, S.C., Wolk, A., 2012. Red and processed meat consumption and risk of pancreatic cancer: meta-analysis of prospective studies. *Br. J. Cancer* 106, 603–607.
- Larsson, S.C., Bergkvist, L., Wolk, A., 2006. Processed meat consumption, dietary nitrosamines and stomach cancer risk in a cohort of Swedish women. *Int. J. Cancer* 119, 915–919.
- Lijinsky, W., 1987. Structure-activity relations in carcinogenesis by N-nitroso compounds. *Cancer Metastasis Rev.* 6, 301–356. doi: 10.1007/BF00144269.
- Lin, I.N.C., Gruenwedel, D.W., 1990. Mutagenicity and cytotoxicity of N-nitrosothiazolidine-4-carboxylic acid. *Food Addit. Contam.* 7, 357–368.
- Linseisen, J., Kesse, E., Slimani, N., Bueno-de-Mesquita, H., Ocké, M., Skie, G., et al., 2002. Meat consumption in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohorts: results from 24-hour dietary recalls. *Public Health Nutr.* 5, 1243–1258.

- Massey, R.C., Key, P.E., Jones, R.A., Logan, G.L., 1991. Volatile, non-volatile and total N-nitroso compounds in bacon. *Food Addit. Contam.* 8 (5), 585–598.
- Negishi, T., Shiotani, T., Fujikawa, K., Hayatsu, H., 1991. The genotoxicities of N-nitrosamines in *Drosophila melanogaster* in vivo: the correlation of mutagenicity in the wing spot test with the DNA damages detected by the DNA-repair test. *Mutat. Res.* 252, 119–128.
- Pedersen, A.N., Fagt, S., Groth, M.V., Christensen, T., Biloft-Jensen, A., Matthiessen, J., et al., 2010. Danskernes Kostvaner 2003–2008. ISBN: 978-87-92158-67-3. <www.food.dtu.dk> (accessed 27.10.14).
- Penttilä, P., Rasanen, L., Kimppa, S., 1990. Nitrate, nitrite, and N-nitroso compounds in Finnish foods and the estimation of the dietary intakes. *Z. Lebensm. Unters. Forsch.* 190, 336–340. doi:10.1007/BF01184503.
- Peto, R., Gray, R., Brantom, P., Grasso, P., 1984. Nitrosamine carcinogenesis in 5120 rodents: chronic administration of sixteen different concentrations of NDEA, NDMA, NPYR and NPIP in the water of 4440 inbred rats, with parallel studies on NDEA alone of the effect of age of starting (3, 6 or 20 weeks) and of species (rats, mice or hamsters). *IARC Sci. Publ.* 627–665.
- Rohrmann, S., Overvad, K., Bueno-de-Mesquita, H.B., Jakobsen, M.U., Egeberg, R., Tjønneland, A., et al., 2013. Meat consumption and mortality – results from the European Prospective Investigation into Cancer and Nutrition. *BMC Med.* 11, 63.
- Santarelli, R.L., Pierre, F., Corpet, D.E., 2008. Processed meat and colorectal cancer: a review of epidemiologic and experimental evidence. *Nutr. Cancer* 60, 131–144. doi:10.1080/01635580701684872.
- Scientific Committee on Consumer Safety, 2012. Opinion on nitrosamines and secondary amines in cosmetic products. SCCS/1458/11.
- Sen, N., Baddoo, P., Seaman, S., 1993. Studies on the occurrence and formation of 2-(hydroxymethyl)-N-nitrosothiazolidine-4-carboxylic acid (Hmntca) and 2-(hydroxymethyl)-N-nitrosothiazolidine (Hmnhz) in various cured smoked meats, fish and cheese. *J. Sci. Food Agric.* 61, 353–356. doi:10.1002/jsfa.2740610311.
- Shank, R.C., 1975. Toxicology of N-nitroso compounds. *Toxicol. Appl. Pharmacol.* 31, 361–368. [http://dx.doi.org/10.1016/0041-008X\(75\)90257-4](http://dx.doi.org/10.1016/0041-008X(75)90257-4).
- Su, T., Ding, X., 2004. Regulation of the cytochrome P450 2A genes. *Toxicol. Appl. Pharmacol.* 199, 285–294. <http://dx.doi.org/10.1016/j.taap.2003.11.029>.
- Tricker, A.R., Kubacki, S.J., 1992. Review of the occurrence and formation of non-volatile N-nitroso compounds in foods. *Food Addit. Contam.* 9 (1), 39–69.
- Tricker, A.R., Preussmann, R., 1991. Carcinogenic N-nitrosamines in the diet: occurrence, formation, mechanisms and carcinogenic potential. *Mutat. Res.* 259, 277–289. doi:10.1016/0165-1218(91)90123-4.
- Tricker, A.R., Pfundstein, B., Theobald, E., Preussmann, R., Spiegelhalder, B., 1991. Mean daily intake of volatile N-nitrosamines from foods and beverages in West Germany in 1989–1990. *Food Chem. Toxicol.* 29, 729–732. [http://dx.doi.org/10.1016/0278-6915\(91\)90180-F](http://dx.doi.org/10.1016/0278-6915(91)90180-F).
- Umamo, K., Shibamoto, T., Fernando, Y.S., Wei, C.I., 1984. Mutagenicity of 2-hydroxyalkyl-N-nitrosothiazolidines. *Food Chem. Toxicol.* 22, 253–260.
- Zeilmaker, M.J., Bakker, M.I., Schothorst, R., Slob, W., 2010. Risk assessment of N-nitrosodimethylamine formed endogenously after fish-with-vegetable meals. *Toxicol. Sci.* 116, 323–335.