Annex to: Risk assessment of *N*-nitrosamines in food. doi: 10.2903/j.efsa.2023.7884

Annex F – Outcome of the public consultation

F.1 Rationale for the public consultation and summary of its outcome

In line with EFSA's policy on openness and transparency, and for EFSA to receive comments on its work from the scientific community and stakeholders, EFSA engages in public consultations on key topics. Accordingly, this draft opinion together with its annexes was released electronically for public consultation from 12 October until 22 November 2022 by means of an e-submission tool. The comments were made publicly available immediately after the closure of the public consultation in OpenEFSA[1]

Comments were received in the electronical tool from 12 interested parties from 8 countries and EU. One interested party didn't use the electronic tools and provided information via e-mail within the requested deadline. Table 1 provides an overview on the interested parties that have submitted comments through the electronic submission.

Table 1: Overview on stakeholder comments received

Stakeholders	Category	Country
Sodin David	Private	UK
Coudray Guillame	Private	FR
Zhou Pingping	Private	CN
Farrè Maria José	Private	SP
Arozamena_Ramos Eduardo	Private	SP
Ellutia Ltd	Private sector (e.g. industry, consultancy, etc)	UK
UK Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT)	Public sector (e.g. industry, consultancy, etc)	UK
German Federal Institute for Risk Assessment (BfR)	National Authority	DE
French Agency for Food, Environmental and Occupational Health & Safety (Anses)	National Authority	FR

1 https://open.efsa.europa.eu/consultations?search=nitrosamines

Rijksinstituut voor Volksgezondheid en Milieu (RIVM)	Research Institute	NL
European Medicines Agency (EMA)	EU agency	EU
CLITRAVI	Private sector (e.g. industry, consultancy, etc)	BE

F.2 Assessment of comments and use for finalisation of the opinion

The comments received were duly evaluated by the EFSA WG on *N*-nitrosamines in food and wherever appropriate taken into account for the finalisation of the draft opinion. Table 2 provides a detailed list with all comments received from interested parties together with EFSA responses and explanations how the comments were considered in the final opinion opinion. Some comments, especially those suggesting editorial changes, have been directly addressed in the text of the opinion, if they were considered appropriate. Identical comments that were submitted by one stakeholder twice or more are included only once in Table 2.

Table 2: Stakeholder comments and EFSA responses

Stakeholder	Comment number	Chapter	Comment	EFSA response
Sodin David	1	3.1.4.3. The mutagenic and carcinogenic potency of *N*-NAs	Since chemical and biological reactions occur on a molar basis, surely it is strongly preferable to express relative carcinogenic potencies as molar TD50s. This concept was considered fundamental by Bassan et al in their 2011 report to EFSA on the TTC (sp.efsa.2011.EN-159.pdf). Using weight values for TD50s when performing read-across (particularly when NDMA and NDEA are used as reference compounds) can lead to significant overestimates of the carcinogenic potencies of *N*-nitrosamines with higher molecular weights.	When performing mechanistic investigations with the extra-thermodynamic (Hansch) QSAR approach, potencies should be better expressed in molar terms. However, Read Across is a less demanding, yet robust approach, and potencies can be expressed in weight. In addition, use of weights is common to the different toxicity studies (acute, long-term, BMD, etc) and to exposure measurements. To be consistent and comparable, it is absolutely necessary to express every measure in the same way. It should also be noted that the differences in molecular weight of the n-nitrosamines of interest in this opinion are relatively small, involving only a few atoms (see, for example, the structures of NDMA, with MW = 74.1, and NDBA, with MW = 158). Large differences would be present in the case many rings were involved (which is not the case here).

	2	3.1.2.5.1. Predicting carcinogenicity potential for *N*-NAs without animal carcinogenicity data	I agree that data from the Ames assay provide a high level of predictivity for *N*-nitrosamine carcinogenicity (see Trejo-Martin et al, 2022). Unfortunately, some major health agencies refuse to accept the results of a well-conducted Ames assay as evidence for the likely non-carcinogenicity of an *N*-nitrosamine (see Glowienke et al, 2022). NCTR in the USA has investigated modifications to the Ames assay protocol in order to minimise the possibility of false-negative results (see Heflich 2022 - file more than 8 MB; can be sent separately if interested). Some of the recommendations regarding Ames testing are problematic; for example the recommended low solvent volumes have been shown to be insufficient to dissolve some *N*-nitrosamines at appropriate concentrations.	There is general agreement in the published literature that the Ames test predicts the carcinogenic activity of *N*-nitrosamines. Recent examples also include analysis of impurities in pharmaceutical drugs (Thresher et al, 2020; Trejo-Martin et al., 2022). The above references have been added in Section 3.1.2.5.1.
	3	3.1.2.5. *N*-NAs carcinogenicity; the structure-activity relationships	Multiple publications and presentations are available on SARs for *N*-nitrosamines. Researchers involved with *N*-nitrosamines in pharmaceuticals tend to focus on the impact of structure on metabolic activation. Examples are attached. (PDF available in TEAMS HERE)	Thanks for the indication. The list of references has been updated in Section 3.1.2.5.1 with the references Cross and Ponting, 2021; Dobo et al, 2022; Thomas et al, 2022.
Guillame Coudray	4	5. Recommendations	One must commend the CONTAM panel for this very extensive examination of the nitrosamines issue. It is really a remarkable Draft report. Yet I should note that the "recommendations" section is not totally convincing. In essence, the CONTAM panel recommends to conduct "more studies". While more studies can always me useful, one should not forget that there is also an urgent need for public health prevention. Hence, CONTAM panel shall recommend that resolute action is taken to mitigate the risk posed by exposure to nitrosamines. One shall not forget that in some parts of EU, notably in the Balkans, colorectal cancer reaches very high prevalence. (see https://gco.iarc.fr/). EFSA needs to take resolute action on this topic and cannot simply recommend further research. This evaluation is a great step in the right direction, but its recommendations shall be in line with its findings. The panel should recommend: -to revise present ADI which, as said above, have been based on uncomplete knowledge - to conduct a full evaluation of other nitroso compounds NOT limited to nitrosamides but specifically addressing nitrosyl-haem and more globally, "the fate of NO and	Indeed colorectal cancer and the role of substances other than *N*-nitrosamines deserves scientific investigations. EFSA has been requested to assess the risks for public health related to the presence of *N*-nitrosamines as contaminants in food. Consequently, the EFSA CONTAM Panel assessed the risk to public health related to the presence of *N*-nitrosamines as contaminants in food matrices prior to consumption. This assessment excludes (i) compounds other than *N*-nitrosamines and (ii) *N*-nitrosamines which are formed endogenously after consumption of food. Nitrosyl-haem does not meet the criteria for the present opinion. Regarding the endogenous formation of *N*-nitrosamines, as explained in

			haem" 1 during processing 2 during digestion 3 in the colon CONTAM panel shall stress the fact that non-nitrated meat products are already available. As far as dry-cured products are concerned (such as Parma ham) it is important to note that Zinc Protoporphyrin is present instead of nitroso-compounds. More generally, it would now appear necessary to question the need to use nitrate or nitrite in meat processing. For this purpose, I hereby attach a Codex Alimentarius 2017 document that clearly states that numerous other meat-processing technologies are available. Thank you.	section 1.3.7., the ANS Panel (EFSA ANS Panel, 2017) quantified the theoretical amount of *N*-nitrosodimethylamine (NDMA) upon digestion of nitrite at the level of the ADI (0.07 mg/kg bw, nitrite ion per day). Applying a number of conservative assumptions, the Panel estimated that the margin of exposure (MoE) would be much greater than 10,000 and therefore of low concern (EFSA, 2005; EFSA Scientific Committee, 2012a). Therefore, also the recommendations do not address nitroso compounds other than *N*-nitrosamines. A clarification on this point has been included in section 1.2 interpretation of the terms of reference.
	5	4. conclusions	**lines 5700-5701** Question: Regarding the reason why no Reference Point can be established: is it really due to limitations in study design? Or due to the intrinsic difficulties for setting a Refence Point for carcinogenic substances? **lines 5718-5721:** It would be needed to provide the data separately for processed meat and for unprocessed meat. If the data does not allow to make this distinction, it should be explained. The fact that unprocessed meats do generate endogenous nitrosation has been stated before in the report, but it has not been said that the levels of presence of non -endogeneous nitrosamines are equivalent. Hence, whenever data is available, it is needed to distinguish processed/unprocessed meats.	**Lines 5700-5701:** The reason that the epidemiological studies on *N*-nitrosamines cannot be used to establish a reference point is due to the limitations in the study design. The details are outlined in section 3.1.3.1. **Lines 5718-5721:** The sentence was slightly modified in the text to show that samples from colorectal cancer patients consuming red meat, considered unprocessed or processed, carry the same mutational signatures. With regard to endogenous nitrosamine formation please see comment 4.
	6	3.1.12.2. Hazard identification and characterisation	As I stated repeatedly above, there is a need for more clarity as regards the other nitroso compounds that have not been included, especially nitrosyl haem. More generally, there is a severe lack of considerations regarding haem. Issues concerning haem shall be much	Please see comment 4 above.

			more present in the evaluation of nitroso compounds in cured meat products (and uncured meat products, obviously). The Report from Belgian Superior Health Council 2013, already mentioned, gave an excellent overview. A minima, it would appear that CONTAM panel should at least say as much regarding haem and its relation to nitrosamines.	This reference concerns processed and unprocessed meat in general. *N*-nitrosamines as such are not discussed in the publication mentioned.
	7	3.1.11.1. Risk characterisation	Very clear. As a careful observer of the scientific controversies around nitroso compounds, I shall warn the CONTAM panel to be extremely warry of possible attempts from private interests to downgrade this evaluation of the risk. It should be noted that in the past, some industry interests have played an important role in downgrading NTP 2001 evaluation Toxicology and carcinogenesis studies of sodium nitrite (CAS NO. 7632-00-0) in F344/N rats and B6C3F1 mice (drinking water studies), Natl Toxicol Program Tech Rep Ser. 2001 May;495:7-273. CONTAM panel members shall not believe that the stakeholders that have been denying carcinogenicity of nitrate-treated and nitrite-treated meats for the last 40 years will not try to spin the results of the present draft opinion. It is the social responsibility of CONTAM panel members to make sure that the conclusions of the opinion are indeed brought to the knowledge of the public and the regulators in an unbiased, untainted fashion. Science does not exist in a vacuum, CONTAM panel members shall postulate that producers and marketers of nitrate-treated and nitrite-treated meat products will go to great length to try to minimise or neutralize the impact of present draft opinion.	Independence, transparency and openness are the cornerstones of EFSA's work. EFSA applies a robust set of internal mechanisms and working processes to safeguard the independence of its work. EFSA's independence policy is among the most robust and comprehensive within the EU agencies, as also recognised by the EU Ombudsman. EFSA's work is scrutinised by a number of institutions and stakeholders, including the European Court of Auditors, the European Parliament, the European Ombudsman, and civil society.
	8	1.3.7. Previous assessments	It might be interesting to go as far as SCF 1990 and SCF 1995 "expert opinions": « Nitrates and nitrites », Reports of the Scientific Committee for Food, Vingt Sixième série, 19 octobre 1990, Commission européenne / Directorate-General Internal Market and Industrial Affairs, 1992 (p. 24-25) « Nitrates and nitrites », Reports of the Scientific Committee for Food, septembre 1995, Trente Huitième série, 22 septembre 1995, Commission européenne, 1997. As frequently stated, EFSA 2003 and EFSA 2017 reports and evaluations worked on the premise that SCF 1990 and SCF 1995 reports were fair and accurate, and that the ADI derivation now in use was fundamentally sound. Was it	Currently, the ADI as calculated by the EFSA ANS Panel (2017) is the only health base guidance value available to estimate the risk to nitrate and nitrite in food. A recent evaluation of ANSES (2022), confirmed this conclusion. EFSA is revising its opinions considering the availability of new scientific evidence that would support such a revision. Please note that the present opinion concerns the

			known, at the time, that 1995 chair Mr Gerard Pascal was a high-ranking officer at ILSI, the food-industry group known for meddling in food-additives regulation and promote the interests of international food corporations, including nitrited-meat processors? See https://ilsi.eu/cv-gerard-pascal/ On ILSI please see Steele ans al., "Are industry-funded charities promoting "advocacy-led studies" or "evidence-based science"?: a case study of the International Life Sciences Institute", Global Health, 2019 Jun 3;15(1):36. doi: 10.1186/s12992-019-0478-6. Today, Mr Pascal is still at work fiercely defending nitro-meat industry interests, as we have told in this 2020 paper: https://reporterre-net.translate.goog/Dure-bataille-pour-liberer-la-charcuterie-des-nitrites-cancerigenes?_x_tr_sl=auto&_x_tr_tl=en&_x_tr_hl=fr&_x_tr_pto=wapp Most notably, SCF1995, under Mr Pascal guidance, stated (p 19, see attached) that "overall, extensive epidemiological studies on nitrate have failed to demonstrate an association with cancer risk in man. The Committee therefore felt it appropriate to derive an ADI." As it appears that 2022 CONTAM panel would NOT agree with that bizarre 1995 assertion, I can only hope that EFSA will quickly put an end to the inaccurate ADI that is in place.	risk assessment of *N*-nitrosamines only and not of nitrate and nitrite.
	9	1.3.5. Mitigation measures to reduce the *N*-NAs concentration in food and drinking water	This is a most important paragraph. The CONTAM group does an excellent job at retracing the history of ascorbates and erythorbates. It mentions USDA but it would make sense to stress that this practice is now standard in EU too. This takes the form of added vitamin C in the form of sodium ascorbate (E301 on the label) or erythorbic acid (E315, also called isoascorbic acid), or sodium erythorbate (E316). It could be added that this is why these "E" (for European) numbers are very often found on the labels of nitrated and nitrited meat products. But another aspect of this issue MUST also be addressed. Namely, the fact that adding vitamin C hinders nitrosation BUT promotes nitrosylation. As Dr Océane Martin writes, "the addition of ascorbic acid, in order to avoid the formation of carcinogenic nitrosamines, will promote nitrosylation reactions and therefore form a bridge between the nitrite ions and the iron atom of the heme, which leads to the formation of nitrosylated heme". Another researcher, Dr	The text has been revised as follows to address the comment: "For the same purpose, in Europe both ascorbic acid/ascorbates (E300-E302) and erythorbic acid/sodium erhytorbate (E315-E316) are listed in the food additives list of regulation No. 1333/2008/EC. Indeed, their addition is usual in processed meats with added nitrite/nitrate." The use of these compounds accelerates the chemical conversion of nitrite to nitric oxide, inhibiting the *N*-nitrosamine formation (Archer et al., 1975; FAO/WHO, 2019). However, nitric oxide could also promote other types of reactions e.g nitrosylation and

			Nadia Bastide, similarly notes: "The presence of ascorbic acid in cooked meats favours nitrosylation reactions. This is why nitrosylated heme is found in large quantities." quoted from Oceane Martin Promotion de la cancérogénèse colorectale par le fer héminique des viandes: Prévention nutritionnelle, rôle du microbiote et de l'inflammation, Université de Toulouse, 2015, p. 41 ; Nadia Bastide, Fer héminique et cancérogénèse colorectale. Etude des mécanismes et recherche de stratégies préventives, Université de Toulouse, 2012, p. 36-37 There is a large amount of experimental evidence on this topic, and it appears to me that here again, CONTAM panel may not be silent on nitrosylation-related aspects of the nitrosation issue.	formation of potentially toxic compounds (Kostka 2020).
	10	1.3.4.1. Food	The presentation of *N*-Na formation consecutive to heat exposure is excellent and it is it is extremely relevant to the topic at hand and in terms of public health. Some more figures could be added. ANSES 2022 report might be used as a guidance on that specific topic. For example, ANSES states that the highest levels of nitrosamines were found on the one hand when cooking at 150°C, and on the other hand in fried bacon fat (35.6 g kg-1) and in fried pork fat (25.9 g kg-1). (please see ANSES, Évaluation des risques liés à la consummation de nitrates et nitrites, Juillet 2022, p. 44). It seems important that this part regarding nitrosamine formation should be stressed in the general recommendations of the CONTAM panel. It is most relevant in terms of public health for 450millions European consumers. The fact that boiling and microwaving are the safest way to cook nitrite-treated bacon or sausages should be the purpose of an ambitious, high-impact public health campaign targeted at European consumers who (rightfully so) are keen sausage & bacon lovers and barbecuers. Especially, pregnant mothers should be careful when cooking at high temperatures (frying, grilling, baking or barbecuing) sausages and other meat products that have been treated with nitro-additives (nitrate and/or nitrite). Also CONTAM report could be more precise as far as specific cases of *N*-NAs formation due to heat. Notably, it seems essential to mention here that Nitrosamines have often been detected in pizza "toppings" such as nitrite-	Although the role of temperature is significant in *N*-NAs increase after cooking, it is not the only parameter which affects the final concentration. The statement about higher formation of *N*-NAs in grilled and fried products is only based on the literature evidence, and the comments are not peremptory. Indeed, the "collection of data on *N*-NAs in processed foods…….and of cooked products with and without the addition of nitrate and nitrite" is recommended in this opinion in the recommendations section.

			cured salami, nitrite-cured ham or nitrite-cured chorizo, and so on, when the pizza is heated or reheated at 130°C (or 150° in some reports). See specifically H. Deierling et al., « Nitrosamine in Lebensmitteln », Lebensmittelchemie, 51, 1997; Karl-Otto Honikel, « Use and control of nitrate and nitrite for processing of meat products », Meat Science, vol. 78, 2008, (p.75). I attach Honikel, who at the time was a paid advisor to european nitro-meat lobby "CLITRAVI".	
	11	1.3.3. Sources of *N*-NAs in food	In the first sentence, "possibly due to the use of nitrites as preserving agents" is quite far fetched. Rather than this dubiously dubious way of putting it, I believe a reference to the 1 as William Lijinsky would be more appropriate, given the fact that CONTAM expert group often refers to Lijinsky results. I attach the 1999 Lijinsky paper *N*-Nitroso compounds in the diet (https://pubmed.ncbi.nlm.nih.gov/10415436/). Even the first sentence of the abstract shows that the use of "possibly" here is incorrect. (Lijinsky's abstract starts like this: "*N*-Nitroso compounds were known almost 40 years ago to be present in food treated with sodium nitrite, which made fish meal hepatotoxic to animals through formation of nitrosodimethylamine (NDMA)"). The rest of this 24 years old paper is most enlighting. It would be a pity if the CONTAM panel would decide not to take into full account 50+years of accumulated science on the role of added nitrate and nitrite in elevating the level of in-product nitrosation and nitrosylation and endogenous nitrosation (and nitrosylation)	The sentence has been modified according to the suggestion, also adding some other parameters affecting the *N*-NAs formation in cured meats.
	12	1.3.1. Chemistry	As far as definitiion of *N*-Nitrosamines is concerned, there is here a need for more clarity as regards what exactly are "nitroso compounds". The first sentence of the "Chemistry" paragraph starts by the word "nitroso compounds", but the rest mostly defines nitrosamines. In the "Extensive literature search on *N*-nitroso compounds in food" published by EFSA on october 7 2022 (https://www.efsa.europa.eu/en/supporting/pub/en-7583) it is wrongly stated that "nitroso compounds" should be construed as nitrosamines and nitrosamides. If such (bizarre) definition is retained, it is necessary to express clearly why other nitroso-compounds are excluded, notably nitrosyl-haem and nitroso thiols. for illustration I attach De la Pomelie 2017 that nicely stresses the	Please note that the first sentence of the "Chemistry" paragraph starts by the word "*N*-nitroso compounds". The initial mandate concerned only nitrosamines. The EFSA's CONTAM Panel asked whether this mandate could be extended to other *N*-nitroso compounds as reported in the minutes of its open 111th CONTAM Plenary meeting under item 9 on new mandates. EFSA launched a call for an extensive literature review to all *N*-nitroso compounds. Due to the extensive literature available on *N*-

			importance of nitrosyl compounds and their interplay with other nitroso compounds. As De la Pomelie and all states, "The first risk of nitrite is due to its reaction with dietary secondary amines during food processing or gastrointestinal digestion to form nitrosamines, some of which are mutagenic. This risk has long been described and it has been well documented in the literature. The second risk of nitrite is due to its reaction with the heme iron of myoglobin to form nitroso-myoglobin. Nitroso-myoglobin gives the dark red color characteristic of raw cured meats. During thermal processing globin denatures and detaches, leading to the formation of nitroso-ferrohemochrome which gives the pink color characteristic of cooked cured meats. These two pigments can release nitrosylheme during heating and digestion. The mutagenicity of nitrosylheme has recently been reported."	nitrosamines and the lack of data for other *N*-nitroso compounds the Panel addressed the initial mandate. Re-evaluation of nitrates and nitrites or risk assessment of other nitroso compounds such as nitrosyl iron was out of the scope of this evaluation. Indeed colorectal cancer and the role of substances other than *N*-nitrosamines deserves scientific investigations.
	13	1.3. Supporting information for the assessment	The CONTAM report is most interesting as regards previous assessment. Yet I suggest that the report reference (or quote) the quite extensive 2013 report by Belgian Superior Health Council, who also made and encompassing , rigorous description of the state of scientific knowledge 10 years ago, and its implication for public health.	The 2013 report by Belgian Superior Health Council is a comprehensive evaluation on red meat, processed red meats and the prevention of colorectal cancer. However, the summary of previous assessments in the opinion refers to evaluations who provide risk characterisation of *N*-nitrosamines in like with the mandate.
	14	1.2. Interpretation of the terms of reference	It would be good to state here that there is a good deal of ambiguity as far as what exactly "nitrosamines" are. For most non -experts (and for some experts), nitrosamines and nitroso-compounds are synonymous. It is a fundamental rule of evaluations that ambiguities of vocabulary must be clearly stated and clarified. See for example Voir Sandrine Fraize-Frontier (ANSES), « Analyse d'incertitude », in Valérie Camel et al., Risques Chimiques liés aux aliments, Lavoisier, 2018, p. 80.	The first paragraph of the section 1.3.1 on Chemistry has been modified to clarify this point. The classification of *N*-nitroso compounds has been added together with the reason why only *N*-nitrosamines have been considered.
	15	1.1.1. Background and rationale of the mandate	There is a need to clarify the fact that it is well known that nitrosation AND nitrosylation are at the center of the issue. Please find attached a page (in French only, I'm afraid) of Fabrice Pierre's 2016 in Viandes et produits carné. Dr Fabrice Pierre's work is largely funded by French and European nitrited-meat-processors, this synthetic presentation is excellent at explaining why it would be aberrant for EFSA to disregard nitrosyl-haem when	Please refer to the comments 4 and 12 above.

			considering nitroso-compounds. full reference is as such: https://www.viandesetproduitscarnes.fr /phocadownload/vpc_vol_32/3245_pierre_produits_carnes_et_risque_cancer.pdf)	
	16	1. Introduction	An excellent introduction. The CONTAM expert group must be commended for the refreshing clarity of the evaluation. It is most enlighting to understand that the expert group responsible for previous (2017) evaluations took the risk only to evaluate endogenous nitrosation. I think it would make sense, in order to properly introduce the topic, to stress that this issue has been at the center of scientific and political discussions and oppositions for nearly 70 years (if we take Barnes and Magee 1954 as starting point) or more accurately for 50 years (if we take LANCET 1968 editorial attached, unsigned but written by William Lijinsky). As a student of the political history of nitrite, nitrate and nitroso-compounds regulation, I believe that many of the topics already observed in 1968 are still relevant today.	Thank you for your interesting comment. EFSA's opinions do not enter into political and historical aspects but are focusing on the evaluation of the scientific evidence available. However, it is evident that a large part of the scientific literature assessed dates back to 1960s and 1970s.
	17	Summary	The 32 *N*-NAs are nicely defined. The work of the CONTAM group must be commended for its very high quality and for its extensivity as regards *N*-Nas. The summary gives a clear, well written overview of the issue at hand and of why there is a need to evaluate risks associated with *N*-NAs. Yet, as I stated above, there is widespread confusion in the public, among decision makers in the press and sometimes also among scientists, as regards the distinction between "*N*-nitrosamines" and "nitroso compounds". Therefore would be appropriate to stress in the summary that *N*-nitrosamines are an important group of nitroso compounds but that they constitute only part of the nitroso-compounds that are likely to be genotoxic and induce tumors in rodents. Therefore I believe it would be essential, in the summary, to mention the question of the other nitroso-compounds that are likely to play a role in carcinogenesis . why not, already at this stage, mention nitrosamides, nitrosyl-haem and nitroso thiols? I attach same file as for Abstract section,(part of latest ANSES report on risks associated with nitrate and nitrite and nitroso compounds, https://www.anses.fr/fr/system/files/ERCA2020SA0106Ra.pdf, pages 45-47). I apologize for being able to provide only an automatic translation to English	Thank you for your comment. Please see also the comment 12 and 14. The summary section includes a summary of the risk assessment presented in this opinion.

	18	Abstract	There is widespread confusion in the public, among decision makers and sometimes among scientists, as regards the distinction between "*N*-nitrosamines" and "nitroso compounds". Therefore would be appropriate to stress in the abstract that *N*-nitrosamines are an important group of nitroso compounds but that they constitute only part of the nitroso-compounds that are likely to be genotoxic and induce tumors in rodents. I believe it should be good, right at this stage, to mention the question of nitrosyl-haem (see ANSES report on risks associated with nitrate and nitrite and nitroso compounds, https://www.anses.fr/fr/system/files/ERCA2020SA0106Ra.pdf, notably pages 45-47) of which I attach a few relevant pages) . See also for example Diane de La Pomélie et al., "Mechanisms and kinetics of heme iron nitrosylation in an in vitro gastro-intestinal model", Food Chemistry, 239, January 2018, and Santarelli et al, Meat processing and colon carcinogenesis: cooked, nitrite-treated, and oxidized high-heme cured meat promotes mucin-depleted foci in rats, Cancer Prev Res (Phila), 2010	The abstract includes a short synthesis of the risk assessment presented in the opinion.
Pingping Zhou	19	3.1.6. Dose-response analysis	This is confusing as why was the study cited in the WHO drink water assessment document (peto 1991) not used for the BMD calculation? It is recommended that the Peto, 1991 study literature be selected for BMD modeling to derive the BMDL10. The reasons are 3 as follows: 1) the literature is relatively new compared to Brantom et al. 1983 study. 2) the number of animals tested is large. 3) the derived BMDL10 is small and consistent with the population protection principle.	Peto used the data from Brantom´s PhD thesis for the publications "Dose and time relationships for tumor induction in the liver and esophagus …" and "Effects on 4080 rats of chronic ingestion of *N*-nitrosodiethylamine or *N*-nitrosodimethylamine…", both appearing in Cancer Research in 1991. In the first publication Weibull analyses were used to calculate dose and time relationships for the effects of chronic exposure to 15 different dose levels of NDMA or NDEA. Peto calculated a lifelong risk in percentage per µg *N*-NA/kg bw/day. In the second publication, Peto described that the product between the dose x median time to death is relatively constant, considering additional factors such as very low and high dose levels, specific *N*-NAs studied and different tumour entities. A

				BMDL10 was not derived in these two publications. The data were given in a very summarized way. The data on the tumour occurrence are documented in more detail in Brantom´s PhD thesis. To our understanding, the data from Brantom´s PhD thesis were part of the derivation of a guideline value by WHO in 2002 and 2008.
	20	2.1.1.2.Concentration data for *N*-NAs in food	The occurrence data of *N*-NAs on foods used for dietary exposure assessment span too long a period, with data submitted from four EU member states from 2003-2021 and from the literature from 1990-2021, more than 20-30 years, is it appropriate to use for the current population risk assessment? The most important content data either raw data or literature data, there is no requirement for analytical methods in the occurrence data quality criteria. Analytical methods for *N*-NAs include concentration, followed by chromatographic separation of the components in the extract and detection of *N*-nitrosamines. The detection and analysis process of artificial formation and small molecule mass spectrometry matrix effects are the difficulties. In addition, the level of *N*-NAs in food products possibly due to the influence of precursor amines and nitrosating agents. In particular, *N*-NAs in aquatic products are affected by many factors such as category or species, freshness, process, temperature, and storage conditions. The span of LOD (0.03-0.25 ug/kg) or LOQ of method (0.005-10 ug/kg) for data from different sources is large, varying by 2 to 3 orders of magnitude, which can lead to large uncertainties in exposure results when dealing with left-censored data, and can even affect the order of ranking of the average exposure contribution of different food categories. Risk assessment should be used as soon as possible with a sensitive method to reduce the uncertainty of exposure assessment results.	The uncertainties linked to the available occurrence data were carefully assessed in the uncertainty analysis. The Panel acknowledged that the assessment of P95 exposure was subject to significant sources of uncertainty, which could make the true value up to a factor of three lower or a factor of eight higher than the one provided in the opinion. Lack of appropriate analytical methods and of occurrence data were also acknowledged in the conclusions and relevant recommendations were provided.
Maria Jose Ferrè	21	1.3.4. Effect of processing on the residual concentration of *N*-	In line 791 of the document the following statement is made which is incorrect "Higher *N*-NAs levels, up to 41.5 ng/L of NDMA and 59.1 ng/L of NPYR, were detected by Farré et al. (2020) in Spain and Chen et al. (2019) in China, respectively." Nevertheless the original sentence of	The text has been revised accordingly.

		NAs in food and drinking water	the scientific article, which I am first and corresponding author is: "The maximum concentration of NDMA formation potential measured in raw waters was 41.5 ± 4.3 ng/L". This is a very important difference as the 41.5 ng/L of NDMA is referring to the precursors of this compound (formation potential) and not NDMA itself. Additionally, this concentration of NDMA precursors was found in raw untreated water and not drinking water. We claim the correction of this sentence. The author should clarify that this value refers to formation potential or instead copy the correct sentence of the paper which is "The maximum concentration of NDMA measured in the final treated water or samples taken from the distribution systems was never above 4.2 ± 0.2 ng/L". I am uploading the article with the sentences highlighted in yellow. Thank you.	
Ellutia Ltd	22	1.3.2. Analytical methods	I work for Ellutia a UK company that still produces the TEA detector mentioned in this section. We have conducted a lot of work in the analysis of nitrosamines in both food and other industries such as the pharmaceutical industry and would disagree with the statement on line 508 that mass spectrometry is undoubtedly the best technique. Our experience has been that the TEA detector offers better sensitivity and selectivity for nitrosamine compounds than most GC-MS systems. GC-MS systems often have to be operated in SIM mode for sensitivity which means the system can potentially miss other nitrosamines present. With our recent work with a number of pharmaceutical companies looking at nitrosamine impurities in their product lines, they had been finding issues with false positives and achieving the required detection limits when working with even LC MSMS systems. The TEA detector can also be used with a chemical stripping system for the detection of ATNC (apparent total nitrosamine content) This approach allows the reporting of a value for total volatile and non-volatile nitrosamine content in a single result. This approach was used by premier foods in the UK in a report prepared for the UK government (An Investigation to establish the types and levels of *N*-nitroso compounds (NOC) in UK consumed foods). Since that report was produced further development of total nitrosamine analysis has been undertaken with the recent launch of a new automated approach that allows for much	The text has been modified. No article from 2010 to the present describes the use of TEA as detector for the determination of *N*-NAs in food. This is the reason why this type of detection has not been mentioned in Table 2.

			lower levels of detection than seen in the system used by premier foods in the report mentioned. (https://www.ellutia.com/automated-total-nitrosamine-analysis/) This is an approach that could potentially offer a lot of benefits when looking to screen a large number of samples for potential nitrosamine content so should be considered within the analytical methods. Ellutia would be happy to work with relevant organisations to further evaluate this technique in the food sector.	
COT, UK	23	3.1.7. Benchmark dose modelling	There was an observation by the Committee that the data used seems to be from Brantom's PhD thesis rather than that in the Peto et al papers (in Cancer Research). Peto (who developed the TD50 concept) took into account two extra pieces of information about the liver tumours: firstly, whether the tumour was "fatal" (i.e., killed the animal) or incidental (i.e. was found when the animal died for some other reason such as terminal sacrifice); secondly, the time to tumour incidence). The time to tumour incidence/survival was highly dependent upon the dose level. The BMD (Proast) analysis uses, possibly, the crude tumour count and doesn't take this other information into account. It is not certain how this other information would affect the BMD10 values and it is likely that it would be difficult to analyse because the data are probably no longer available. The BIBRA nitrosamine studies were controversial at the time. Sophisticated modelling of the data (arguably more extensive than the BMD) was undertaken and described in the Cancer Research papers and it was argued that there were carcinogenic effects even at the lowest doses in some of the studies. This probably suggests some caution should be used in relying uncritically on the BMDL10 of mg/kg bw per day.	As mentioned in the comment, the original data on the tumour latency periods are only given in a cumulative way. The shortened latency period might be relevant only for the higher dose range. In addition, BMD (Proast) analysis confined to the low dose range provided BMDL results similar to BMD (Proast) analysis comprising all doses.
	24	Abstract	The UK COT considered this to be a very positive opinion and comprehensive review	Thank you.
BfR, DE	25	4. Conclusions	5509, page 199 "ten carcinogenic *N*-NAs" should be replaced by "10 of the 24 *N*-NAs identified as carcinogenic"	Text modified accordingly
	26	3.1.5.3. Carcinogenicity	Line 4681, page 152 "NTMCA" should possibly be replaced by "NMTCA".	Editorial corrected
	27	3.1.5.2. Genotoxicity	Line 4665, page 152 "NTMCA" should possibly be replaced by "NMTCA".	Editorial corrected
	28	2.2.1. Dietary exposure assessment	Line 1079 ff, page 37 The procedure to calculate the external chronic exposure using the available food consumption and food occurrence data is well described	Exposure to other sources was not within the remit of this opinion. However, based on literature reviews

			here. But as mentioned in other parts of the opinion this is not the only source of dietary exposure and the other dietary sources should be described here as well in line with other EFSA opinions. In particular it should be better described here for which of the consumption data a match to the occurrence data was not possible. Further the possible endogenous formation of nitrosamines should be addressed as possible source for dietary exposure that is not be considered. In general, the concept to start with available data that might underestimate the exposure but posing a risk (MOE<10.000) so that the other sources might not be of relevance to show that there is a risk should be made more clear in this chapter. Similarly, it should be clarified that the approach used here is not able to provide a good estimate for the actual total dietary exposure. Such a statement should also be added to other parts of the assessment (e.g. Risk Assessment or Conclusion)	the exposure to other sources of *N*-NAs was described in the section 1.3.6. The food categories for which data are not available, are listed in the section 3.3.1 and are : "Fruit and fruit products", "Fruit and vegetable juices and nectars (including concentrates)", "Grains and grain-based products", "Legumes, nuts, oilseeds and spices", "Milk and dairy products", "Starchy roots or tubers and products thereof, sugar plants", "Vegetables and vegetable products" and "Water and water-based beverages". The Panel considers it clear that that exposure for any other sources can only add to the risk. The uncertainties linked to the available occurrence data were carefully assessed in the uncertainty analysis and relevant recommendations were provided. See also comment 20.
	29	1.3.4.1.Food	Line 737, page 27 "Herrmann et al." should be deleted	Editorial corrected.
	30	1. Introduction	Table of Contents Line 299-403, page 11-12 Section numberings and headings should be revised throughout the entire document. For instance, a section header "3.2. Exposure assessment" should be inserted before the subsection "Occurrence data". Moreover, "3.1.11. Risk characterization" should be replaced by "3.3. Risk characterization" and "3.1.12. Uncertainty analysis" should be replaced by "3.4. Uncertainty analysis".	Editorial corrected
	31	Abstract	Line 15-17, page 2 The uncommon abbreviation TCNAs should be introduced more clearly. BfR suggests to modify this sentence as follows: "The risk assessment was confined to those ten carcinogenic NAs (TCNAs) occurring in food, i.e.,"	The sentence was revised according to the suggestion.
Anses, FR	32	3.1.12. Uncertainty analysis	**Lines 5404-5405** Those two issues should be reported within the abstract. In this regard, the claim of 98-100% certainty of being below an MoE of 10,000 seems a very	**Lines 5404-5405.** The two major uncertainties i.e. i) the high number of left censored data and ii) the lack

			strong, if not unlikely, conclusion given the very small number of samples quantified.	of data on important food categories were included in the abstract. The Panel's plausible range for their combined impact is a factor of 0.3 to 8, i.e., the Panel considered with at least 98% certainty that the actual P95 exposure of EU toddlers is between 0.3x and 8x the maximum MB estimate. However, even considering the exposure as low as 0.3 times the combined certainty for the final conclusion is very high since this exposure divided with the BMDL (which was judged to be of high certainty) will indicate MoEs lower than 10000. A more detailed description of the calculations used to combine the uncertainty for the hazard and exposure components has been added to the section E.4.3.2 of the annex E. Section 3.5.3 was revised to explain better why the exposure assessment is uncertain. Among all the uncertainties that were identified, the effect of missing food categories, which would lead to higher exposures, was judged to be the most important. This is reflected in the selection of the range of 0.3 to 8 which extends more in the upward than the downward direction.
			Line 5421. Specify the direction of uncertainty (under or over estimate) for each item. In the risk evaluation, worst cases are studied, and it is considered that all *N*-NAs have the same carcinogenic potential as NDEA. On one part, it seems that cancer risk is overestimated in these worst cases.	**Line 5421.** The direction of the main individual sources of uncertainty are indicated in the text. Considering the uncertainties regarding the potencies of the ten carcinogenic nitrosamines, the Panel made conservative assumptions about potency for the reasons given in the Mode of Action section 3.1.4.4. With regard to the possibility of synergistic effects, the Panel

			On the other part, is it sufficient to cover any possible synergistic effects of dietary *N*-NAs? The possible interacting effects of *N*-NAs are not discussed; maybe, future epidemiological studies could be designed to study this point related to interacting effects of *N*-NAs and other carcinogenic compounds present in food (recommendation).	concluded in section 3.5.4, that the MOE for the P95 EU toddler exposure to NDEA is less than 10,000 with at least 98-100% certainty. This conclusion refers to NDEA alone. Later on the same page, it is explained that including the other *N*-NAs can only increase the cumulative risk, regardless of their individual potency, including any possible synergistic effects. Therefore, the Panel concluded with at least the same level of certainty that the MOE for the P95 exposure is less than 10,000 for the sum of all the NAs considered in this assessment. Assessing the possible interaction of different nitrosamines with other carcinogenic compounds in foods in epidemiological studies is an important issue. However, the first step is to evaluate the independent effect on health of single nitrosamines. This and further aspects are now addressed in the updated version of the recommendations.
	33	3.1.11. Risk characterisation	Lines 5258-5262 (mean), Lines 5263-5267 (P95). The tables 23-26 require more comments to our opinion. The comments should indicate that, before any uncertainty analysis: (1) all categories of the population appear at risk as for chronic exposure to food *N*-NAs, (2) toddlers, infants and adolescents appear the most at risk compared to adults in the risk evaluation of food *N*-NAs, according to the two scenarios.	The text has been modified with the text in bold to address the comment: 'Considering the TCNAs, most of the MOEs are lower than 10,000 for both exposure scenarios which raises a health concern **for all age groups. According to the two scenarios the MOEs for toddlers, infants and adolescents are lower compared to other age groups.'**
	34	3.1.10. Dietary exposure assessment for humans	**Table 17.** The methodology used to derive min LB should be described.	**Table 17.** The methodology used to derive the occurrence LB is already described at line 4889 in section 3.1.9.1 of the draft opinion published for public consultation while the methodology to derive the exposure LB is described in section 2.2.1. The

			Lines 5075-5078: "Proxies" are used for certain food categories. It should be verified that this is included in the uncertainties. **Tables 18-21 and graphs 1-3** could be set as annexes. Data from total diet studies should also be considered within such expertise. Overall, other exposure sources of *N*-NAs should be listed, as drinking water, drugs, cosmetics and endogen formation (as described in the risk assessment of nitrates and nitrites). This could be introduced as an exposome assessment perspective.	exposure min LB is estimated across surveys as indicated in the title of each exposure result table (range across surveys). **Lines 5075-5078.** The lack of data in drinking water and overall uncertainty linked to occurrence data including the use of proxies was acknowledged and assessed in the uncertainty assessment (section 3.5.3). **Tables 18-21 and graphs 1-3** The Panel considers the inclusion of these tables and graphs useful for the reader. Exposure to other sources was not within the remit of this opinion. However, based on literature reviews the exposure to other sources of *N*-NAs was described in the section 1.3.6. The Panel considers it clear that that exposure for any other sources can only add to the risk and an exposome assessment is not necessary within the remit of this opinion.
	35	3.1.9. Occurrence data	**Lines 4875-4876**: the number of results per country could be indicated in the parenthesis. **Table 14.** NTCA and NMTCA which are not included in the 10 carcinogenic *N*-NAs considered in the opinion should be deleted and add NMA and NSAR (to show that there are no results in the EFSA database for these 2 *N*-NAs included in the exposure assessment). It could be mentioned here that results with 100% unquantified data are not taken into account in the exposure assessment. **Table 15.** Delete NPRO, NTCA and NMTCA (or mention that these *N*-NAs are not included in the exposure assessment; same for Table 14). Add NDPA and NBPA to show that there is no literature data for these two *N*-NAs.	**Lines 4875-4876.** The number of results per country have been added in parenthesis for clarity. **Tables 14 and 15** list *N*-NAs for which occurrence and literature data were available. The fact that some of these *N*-NAs were not included in the exposure assessment is clearly documented in section 3.2. Footnotes have been added to table 14 and 15 to indicate that the non-carcinogenic compounds where not included in the risk assessment.

			Table 16. This table should be transformed for the same information as in table 14 and 15 but only including the 10 *N*-NAs of interest, the Foodex2 category (level 1), the N, the % LC and the source of the results (EFSA database or literature), only for the results used for the exposure calculations (N>6 and %LC<100%)	In section 3.3 selection criteria are described, such as the exclusion of 100% left censored data at specific Foodex2 levels. **Table 16.** Adding the suggested details will make the table difficult to read. The interested reader can find these details in the annex.
	36	3.1.6. Dose-response analysis	The list of ten *N*-NAs occurring in food (TNCA) is not described before this chapter, except for abstract/summary. It should also be mentioned that this list is meant to evolve according to future occurrence data and/or carcinogenicity studies and/or genotoxicity data for *N*-NAs for which these data are currently lacking.	The information on the ten carcinogenic *N*-NAs is now provided at the end of section 3.1.5. Based on the comment a new recommendation has been added as indicted in bold: **The CONTAM Panel recommends to obtain data on the possible occurrence of carcinogenic nitrosamines in food other than the TCNAs.**
	37	3.1.4.3. The mutagenic and carcinogenic potency of *N*-NAs	This section is a clear summary of notions developed in precedent sections and related to: - bioactivation of *N*-NAs in relation with their structure; - poor correlation between mutation/genotoxicity results and carcinogenicity; - ranking of *N*-NAs potency different according on in vitro (Ames, genotoxicity) and in vivo assays. The feeling is that the document present repetitions. Are these repetitions intentional or could they be avoided?	The purpose of this subchapter is to discuss all possible aspects/endpoints to rank the *N*-NAs according to their potency, i.e. bioactivation, genotoxicity and carcinogenicity. These different aspects were compared to each other, which might appear repetitive. This chapter is important, since it is providing arguments to take a conservative approach to group the 10 carcinogenic *N*-NAs in food, as done in the present opinion.
	38	3.1.4.2. Strength, consistency and specificity of the association of the key events and cancer in humans	**Lines 4397-4399.** The reference of the cohort study showing a significant positive association between a NMDA-contaminated antihypertensive drug and hepatic cancer should be added. **Lines 4410-4425.** "To add to the complexity" could be deleted. Obviously, mechanisms of toxicity and carcinogenicity are complex! Useless to start several sentences in this section with these words.	**Lines 4397-4399.** The reference Gomm et al., 2021 has been added for clarity. For a detailed description and limitations of the study please see section 3.1.3.3 and Annex F.3 **Lines 4410-4425.** The text has been deleted according to the suggestions as it only concerns writing style.

			Line 4437. It would be good to remind the meaning of "exome" in a parenthesis or in a foot note, as "part of the genome consisting of all the exons (that code information)". **Lines 4444-4452.** Suggestion of synthetizing/deleting these lines, because the paragraph on the article by Connor et al 2018 is long (although of interest). The reference of the article will be sufficient to the reader interested in getting details. Moreover, the last sentence (lines 4451-4452) – "human liver cancer has much more complex mutational signatures than that identified in mice exposed to a single DNA" is critical: it is obvious that human exposure to multiple and diverse (pro)carcinogens contaminants will produce much more complex lesions than the ones observed in mice treated with one *N*-NA in lab and controlled experiments.	**Line 4437.** A footnote was added to the text: The exome is composed of all of the exons within the genome. **Lines 4444-4452.** As suggested the text has been shortened to maintain the message and delete details. The Panel prefers to retain the mentioned conclusive sentence since this message might not be obvious to every reader (e.g aflatoxins and aristolochic acid induce specific mutational signatures in human liver cancer) .
	39	3.1.4. Mode of action	General comments - This section details the mutational/genotoxicity signature of the two most experimentally studied *N*-NAs, NDEA and NDMA. - It also summarizes a number of statements already discussed in the precedent sections; it can be questioned on the maintenance of this paragraph recapitulating notions already commented or not. - The section is focused on genotoxicity mechanisms, relatively well-known for a long time. It is agreed that bioactivation and genotoxicity drive carcinogenicity. Yet, epigenetic mechanisms of *N*-NAs cannot be occulted and should be evoked even if they are much less studied than genotoxicity. These epigenetic modifications will contribute to modifications in genomic expression and dysfunction of pathways governed by crucial genes (such as proto-oncogenes, suppressor genes?) with consequences in cancer and transgenerational effects. Line 4326. It seems appropriate to mention in the introduction of 3.1.4. and before detailing genotoxic mechanisms of carcinogenicity in the section 3.1.4. that "Epigenetic mechanisms (DNA methylation, histones modifications, non-coding RNAs) are also implicated in the carcinogenicity of *N*-NAs, effects on development and transgenerational effects; however, epigenetic	We agree that epigenetic modifications play an important role in cancer but these have not been investigated in detail in relation to the carcinogenicity of *N*-NAs. Accordingly, a subchapter has been added to the text.

			mechanisms are much less studied and well-known than genotoxicity."	
	40	3.1.3.1.1. Cancers of the digestive system	**Line 4092.** The sentence should be corrected, because the weak association between dietary NDMA and GI cancer is significant (HR 1,13, CI 1,00-1,28), which is not the case for stomach cancer (HR 1,13, CI 0,81-1,57). **Line 4214.** Regarding the conclusion on lung cancer, it should be added that "Smoking was taken into consideration in the four studies". Indeed, in this subsection smoking status has been reported as a variable in the two studies by De Stefani, but not in Loh et al 2011 and Goodman et al (1992). We have checked this point in the two latter articles.	**Line 4092.** The sentence has been corrected **Line 4214.** Please note that it is not necessary since the information on confounding factors taken into consideration in the statistical analysis are included in the tables for all studies and not only for lung cancer.
	41	3.1.2.5.1.1. *N*-NA carcinogenicity predictions	**Line 3748.** Druckrey (not Druckery) **Lines 3798-3799.** The 11 analogues used to establish the (poor) correlation of carcinogenicity with log Kow should have been cited and the results of the exercise of correlation detailed somewhere. But the best scientific attitude would be not to use such a correlation: if there is no information allowing quantification of a TD50, it would be better to conclude that no TD50 can be calculated due to lack of data. Indeed: lipophilicity of a chemical only reflects its ability to cross biological membranes, i.e., its bioavailability. The EFSA Panel precise in the document that mutagenicity cannot predict quantitatively carcinogenicity, a fortiori lipophilicity! Moreover, why calculating these "predicted TD50s"? First, they will not be used further in the document; second, they will constitute TD50 values of poor quality that may be used not appropriately and considered as official because produced by EFSA. Our proposal is to suppress these "predicted TD50s" approached with LogKow. (NDIPA, NEIPA, NMBA, NMVA, NDIBA).	**Line 3748.** Editorial corrected. **Lines 3798-3799.** NSAR is the only carcinogenic *N*-NA in food for which a TD_{50} is not available. For the purpose of this opinion, the predicted TD_{50} value of NSAR was used only for ranking potencies. The prediction was performed by simple read across with a close analogue. In relation to lipophilicity, it should be emphasised that it is usually a major parameter in QSARs for genotoxic and carcinogenic compounds requiring metabolic activation (Wishnok et al, 1978; Hansch et al. 2001, Selassie et al. 2002).
	42	2.2.1. Dietary exposure assessment	**Line 1081.** The methodology used to derive medium bound (MB) should be described. **Line 1086**. Delete "in". The methodology should specify that food categories in which there are 100% unquantified	**Line 1081.** The methodology used to derive the occurrence MB is described at line 4889 in section 3.1.9.1. of the draft opinion published for public consultation. **Line 1086.** The typo was corrected. The criteria for which food categories with 100% left censored data or with

			values were not considered for exposure calculations, as well as food categories with less than 6 samples.	less than 6 samples were excluded (or taken into consideration only at higher level of the foodex2 classification) is documented at line 4995 in the section relevant to the selection of occurrence data (section 3.1.10.1) of the draft opinion published for public consultation.
	43	2.2. Methodologies	Overall, many of the methodologies are described in detail in Chapter 3, when they should be in this chapter.	Section 2.2 provides a description of the more general methodology applied. Concentration data validation and selection were considered as part of the assessment and therefore are given in section 3.3. A sentence in section 2.1.1.2 has been added to link the two sections.
	44	2.1.1.1.Food consumption data	**Line 1003.** Uncertainties related to the methodology should be explained. **Table 3.** The number of individuals should be added directly in the table, either as an extra colon for n per group or in parenthesis for each country. A brief description of inclusion criteria regarding exposure study should also be displayed.	**Line 1003.** As stated in the opinion details of how the Comprehensive Database is used to assess the dietary exposure to food chemicals are published in a 2011 EFSA Guidance (EFSA, 2011b) including the conservative methodologies used to address standard uncertainties linked to survey methodology and data reporting and representativeness. These uncertainties are listed in appendix G and were deemed of low priority and not significantly impacting the exposure assessment. The uncertainties deemed relevant to this opinion were quantitatively assessed and documented in the uncertainty analysis section 3.5. **Table 3.** Details of the number of individuals per survey, country and age group are provided in Annex C.1 to avoid difficulties to read table 3. Selection criteria concerning occurrence data used in the exposure are described in the section3.3

			Lines 1048-1050. It seems wrong to state that "no data as ever been submitted" since there is a chapter 3.1.9.1. presenting the occurrence data provided to EFSA from Czech Republic, 4876 Denmark, Germany and Hungary between 2003 and 2021.	**Lines 1048-1050.** The statement was clarified and it is now stated that no data were submitted **before 2021**. In 2021, *N*-NAs were put into the priority list and four countries consequently submitted the data.
	45	1.3.7. Previous assessments	**Line 969.** "TD50" are first mentioned in the text there, and should be defined; (even if the definition can be extrapolated by analogy to TD05 defined in line 943). The definition of TD50 is given much further, in 3.1.4. only (too far).	**Line 969.** The definition is now given in section 1.3.7 on previous assessments.
	46	1.1.1. Background and rationale of the mandate	**Line 451.** It is suggested to add that "the risks for public health related to the presence of *N*-nitrosamines endogenously generated from amines and nitrates/nitrites provided by food intake, are not in the scope of the document; therefore, the present document does not deal with the risks for public health of "whole" *N*-nitrosamines".	**Line 451.** This explanation has been now added to the interpretation of the terms of reference. The risks for public health related to the presence of *N*-nitrosamines endogenously generated from amines and nitrates/nitrites provided by food intake, are not in the scope of the document.
	47	Summary	Acronyms should be defined at first mention. **Lines 151-153.** It should be mentioned that the TD50s reported in the abstract are derived from CPDB (Gold et al 1991). In the text (section 3.1.4.) the TD50s derived from the LCDB are reported as improved data (Thresher et al 2019) compared to the ones derived from CPDB. **Lines 175-186.** This paragraph should come sooner in the summary. **Line 243.** Several "main contributor" are listed, a definition of this term should be added as well as an explanation of the list, whether or not there is a contribution gradient?	Please note that this version is still a draft and will be further checked. **Lines 151-153**: The text was changed accordingly. The correlation between the TD_{50} values in CPDB and LCDB is very high (Thresher, 2020). The CPDB database was used in this opinion since it comprises TD_{50} values of more *N*-NAs than LCDB. **Lines 175-186**: This paragraph summarizes the MoA chapter, which is given at the end of the chapter on hazard identification. The summary follows the structure of the opinion. **Line 243.** Clarification was provided that the main contributors refer to the exposure.

RIVM, NL	48	B.5 NPIP	RIVM suggests to re-examine the BMD analysis of NPIP. In this analysis, the dose-response curves level off below 100%. This is not logical, because genotoxic compounds will likely result in 100% tumor incidence at high doses. An explanation could be that sensitive animals were taken from the experiment before developing a tumor (the sample size decreases at high doses), which resulted in an underestimated incidence at high doses (i.e. right censoring). RIVM suggests to perform a BMD- analysis including right censored data. This is possible (if data are available) in the R version of PROAST.	In the Eisenbrand et al (1980) study the levelling off of the dose-response curve was due to the high incidence of oesophageal tumors particularly in the highest dose group. This reduced the median survival time dramatically from about 700 to 392 days. Around day 390 probably not as many liver tumours had developed, when compared to dose groups with a median survival of about 700 days. EFSA performed BMD modelling also for data on NPIP, published by Gray et al 1991. $BMDL_{10}$-$BMDU_{10}$ for any liver tumour was 0.030-0.210 mg/kg bw/day, which is in the same range as the $BMDL_{10}$-$BMDU_{10}$, obtained from the data of Eisenbrand et al (1980), i.e. 0.062-0.213mg/kg bw/day. The data of Eisenbrand et al study were used due to the large number of animals in the dose groups (34-78 animals) compared to 12 animals in Gray et al (1991) study.
	49	4. Conclusions	**Lines 5832-5835**: MOE values ranged, at the P95 exposure (minimum LB-maximum UB), in scenario 1 from 3,242 to 183 and in scenario 2 from 322 to 48, across dietary surveys (excluding some infant surveys with P95 exposure equal to zero) and age groups. remark: The minimum LB MOE at P95 exposure in scenario 1 is 3337 for very elderly according to Table 24. Could EFSA check the MOE values in Table 24 and correct the text or the information in the table? **Lines 5836-5837:** EFSA concluded that the calculated MOEs may indicate a health concern. Could EFSA explain why this conclusion was phrased this way considering that the MOEs are far below the minimal MOE?	**Lines 5832-5835**: The values have been corrected. **Lines 5836-5837:** In agreement with previous opinions of the Panel on genotoxic carcinogens such as aflatoxins, the conclusion was revised to: 'The CONTAM Panel concluded that the calculated MOEs for the TCNAs are below 10,000 in both scenarios which raises a health concern' to make it more clear.

	50	3.1.11.1. Risk characterisation	**Lines 5258-5259**: At the mean exposure, in the scenario 1, the MOEs ranges (maximum-minimum) obtained were from 6108 - 4104 (LB) and from 5371 - 3621 (UB) for NDEA remark: The UB MOE range for NDEA for mean exposure in scenario 1 consists of two maximum MOE values instead of a maximum and minimum MOE value according to Table 23. The highest minimum UB MOE listed in Table 23 is 2512563 for infants. Could EFSA check the table and correct the text or information in the table? **Lines 5259-5260**: For TCNAs MOEs were 9105 - 1483 (LB) and 1089 - 581 (UB). remark: The UB MOE range for TCNAs for the mean exposure in scenario 1 consists of two maximum MOE values instead of a maximum and minimum MOE value according to Table 23. The highest minimum MOE in Table 23 is 465116 for infants. Could EFSA check the table and correct the text or information in the table? **Lines 5258- 5267**. In the risk characterization, EFSA identified MOEs for two different exposure scenarios and for the LB, MB and UB scenario. Could EFSA elaborate which scenario provides the most realistic estimate of exposure? **Lines 5268-5275**. In the risk characterization, EFSA calculated MOEs for TCNA using a conservative and an alternative toxicity scenario. Could EFSA elaborate which of these two scenarios provide the most realistic potency of TCNA? **Lines 5258-5275.** A discussion on which *N*-NAs contributed most the dietary exposure to TCNAs is not included in the opinion. Could EFSA consider including such a discussion?	**Lines 5258-5259**: The text has been corrected **Lines 5259-5260**. The text has been corrected **Lines 5258- 5267.** The presented scenarios provide the best realistic estimates with scenario 2 being more conservative. Both scenarios lead to the same overall conclusion which indicates a health concern. **Lines 5268-5275.** Both scenarios are realistic. The use of a scenario with an indicative potency factor concerning TCNAs did not change the final conclusion. **Lines 5258-5275.** The *N*-NAs that contributed most are given in section 3.3.2 and outlined in the graphs 1-3.
	51	3.1.10.1. Selection of the concentration data from the available data sources used in the dietary exposure assessment	**Line 5041 Table 17** Occurrence ranges of each *N*-NA for the food categories included in the dietary exposure assessment (ug/kg). In Table 17, the minimum and maximum occurrence at FoodEx2 level 1 are grouped for each *N*-NA. Table 6 in Annex C lists the mean occurrence data (LB, MB and UB) that were used in the dietary exposure assessment. As this information is of more interest for the exposure assessment, RIVM would like to suggest to replace Table 17 with a table listing the mean	**Line 5041. Table 17** doesn't show the range across foodex2 level 1 categories but rather more detailed levels chosen to be the most relevant. Some of these categories are not standard foodex2 categories e.g. for meat and fish custom categories "processed" and "cooked unprocessed" were used as of

			concentrations of TNCA (with and without RPFs) for the FoodEx2 level 2 food categories. Such a table would only contain 23 food categories, but would be more informative than the current Table 17.	particular interest based on the two scenarios used for the assessment. These summary categories provide more relevant and easy to read information than the FoodEx2 level 2 food categories. Reference to foodex2 level 1 categories in the header was deleted for the sake of clarity.
			Lines 5117-5130 Tables 18 to 21 present the LB, MB and UB P95 chronic dietary exposures to the individual *N*-NAs and TCNAs across European dietary surveys by age group. These tables contain a lot of information and are not easy to read. To improve their readability, RIVM suggests to replace these tables with a table that lists the mean and P95 exposures to only TNCA (with and without RPFs) for the two scenarios across dietary surveys by age group. For the most relevant single *N*-NAs contributing to the exposure to TNCA, addressed in the result paragraph, a similar second table could be added. These tables would support the general conclusion that the margins of exposure are well below the required 10,000. The exposure results for the remaining individual *N*-NAs are already included in the Annex.	**Lines 5117-5130**. It is understandable that due to the large amount of information the tables are complex. However, results on TCNAs are highlighted in bold in order to be clearly indicated. An additional separation line was added in the table to make it even more clear. It is important that the information related to the *N*-NAs occurring in food is presented in the main text.
	52	3.1.8. Use of BMDL and TD50 data for possible grouping of carcinogenic potency	RIVM suggests to use a more accurate approach for deriving the relative potency factors for the compounds. When liver tumor data in the same species are available for all compounds, EFSA could derive more accurate relative potency factors (RPFs) using the RPF options in PROAST, see for practical applications of this approach e.g.: van der Ven et al. 2022 (https://doi.org/10.1289/EHP9888), van den Brand et al. 2021 (https://doi.org/10.3390/toxins14050303), and Bil et al. 2022 (https://doi.org/10.1289/EHP10009).	As outlined in chapter 3.1.4.4., the ranking of the carcinogenic potency of the *N*-NAs partially differs depending to the parameter used, e.g. TD_{50} according the Gold database, TD_{50} according to the Lhasa database, BMDL10 values, genotoxicity etc It may be assumed that the outcome in the RPF options in PROAST will highly depend on which dataset is used.
	53	3.1.2.6.1. Developmental effects and transplacental carcinogenesis	**Line 3807:** editorial remark In this sentence, the table number is missing.	**Line 3807:** Editorial corrected
	54	3.1.2.4.3. Cyclic and aromatic *N*-NAs	**Line 3614:** The last column of the 10th row in Table 7 states "Unclear fig". Could EFSA explain what is meant with this?	**Line 3614:** It has been clarified that ""unclear fig." means an unclear figure in the paper.

	55	3.1.1.1.4. Cyclic non-volatile *N*-NAs	**Line 2303:** More than 90% of unchanged NTCA or NMTCA was recovered in the 24 h urine along with traces in feces (< 2%)? Editorial remark: RIVM proposes to change the sentence to "More than 90% of NTCA or NMTCA was recovered unchanged in the 24 h urine along with traces in feces (< 2%)".	**Line 2303:** The text was edited to read " More than 90% of the administered NTCA or NMTCA was recovered unchanged in the 24 h urine along with traces in feces (< 2%)"
	56	1.3.1. Chemistry	**Line 481:** Editorial remark In Table 1 numbering is missing in the first column for volatile and non-volatile acyclic *N*-NAs.	**Line 481:** Editorial corrected
	57	Summary	**Lines 258-260:** MOE values ranged (minimum LB-maximum UB at the P95 exposure) in scenario 1 from 3,242 to 183 and in scenario 2 from 322 to 48, across dietary surveys (excluding some infant surveys with P95 exposure equal to zero) and age groups. remark: The minimum LB MOE at P95 exposure in scenario 1 is 3337 for very elderly according to Table 24. Could EFSA check the MOE values in Table 24 and correct the text or the information in the table?	**Lines 258-260:** The value has been corrected throughout the text.
	58	Annex C Occurrence data provided to EFSA and retrieved from the literature that have been used in the exposure assessment	**Table 6:** The values for TCNA NO POTENCY/TCNA WITH POTENCY in column J (LB) and column K (MB) of Table 6 seem to be switched, because MB values should be equal or higher than LB values. Could EFSA correct this and possibly check the calculated exposures. Tables 7 and 8: A large number of P95-estimates in columns I to K of Tables 7 and 8 are equal to zero. RIVM assumes that these P95 estimates were not calculated due to less than 60 subjects per survey and age group or because positive intakes only occurred in the upper 5% of the exposure distribution? Could EFSA, via a footnote, indicate why P95-values equalled zero?	**Table 6:** Thank you for spotting the column switch. TCNAs values in table 6 for MB and LB were moved to the relevant column. No value indicates that surveys with at least 60 subjects were not available. Zero indicates positive intakes only occurring in the upper 5% of the exposure distribution. A footnote has been added.
	59	Annex B. BMD analyses	**Page 384:** list of models Could EFSA correct the table with the expressions of the models as indicated below? This information can be found in the report containing the results generated by the EFSA BMD tool. 1) Probit and logistic models were not applied in the analyses and should be removed from the table. 2) The model expressions are not correct for the following models: Log-logistic, Log-probit, Weibull, Two-stage, Exp model 3, Exp model 5, Hill model 3, and Hill model 5. 3) All model expressions are printed twice.	**Page 384:** The report was revised according to comment.
	60	Abstract	**Lines 17-22:** The in vivo data available to derive potency factors are limited and therefore equal potency of TCNAs was assumed. The incidence of rat liver tumours (benign	**Lines 17-22:** The text was changed to explain that the lowest BMDL was

			and malignant) induced by NDEA, the most potent *N*-NA, was selected to derive a benchmark dose lower confidence limit for a benchmark response of 10% (BMDL10) of 10 g/kg bw per day to be used in a margin of exposure (MOE) approach. remark: EFSA assumes equal potency but also states that NDEA is the most potent TCNA. The latter contradicts the equal potency of all TCNAs. Therefore, RIVM proposes to delete the words "the most potent *N*-NA".	used, and this was the one calculated for NDEA.
Arozamena Ramos Eduardo	61	1.3.4.2. Drinking water	There is some confusion between the NDMA Formation Potential and the real concentration. For example Farré et al (2020). The draft Opinion states: Line #792 "Higher *N*-NAs levels, up to 41.5 ng/L of NDMA and 59.1 ng/L of NPYR, were detected by Farré et al. (2020) in Spain" But this is not correct. These levels are NDMA formation potential, not real NDMA concentration. Farre et al, 2020 report "The actual concentration of NDMA in the final treated water and samples taken from the distribution system was never above 4.2 ± 0.2 ng/L. " Some clarification is needed about this issue.	Corrected (see also comment n.21).
EMA, EU	62	All sections	The EFSA report refers to the European Medicines Agency's (EMA's) Committee for Medicinal Products for Human Use (CHMP) which published an assessment report on nitrosamine impurities in human medicinal products (EMA, 2020). It should be noted that this should be read in conjunction with the question-and-answer document for marketing authorisation holders on implementing the Article 5(3) CHMP opinion. https://www.ema.europa.eu/en/documents/referral/nitrosamines-emea-h-a53-1490-questions-answers-marketing-authorisation-holders/applicants-chmp-opinion-article-53-regulation-ec-no-726/2004-referral-nitrosamine-impurities-human-medicinal-products_en.pdf which includes updates on risk factors for the presence of nitrosamines and on the application of limits for nitrosamines in medicinal products. It should also be noted that the approaches and methodologies used for the risk assessment of nitrosamines in the food and medicines sectors differ for several reasons including the following: • The number of nitrosamines potentially present in food is limited and are most commonly small volatile nitrosamines. While many of the same nitrosamines as in food have been detected in medicines, most nitrosamines	The *N*-NAs in food are considered carcinogenic nitrosamines with calculated TD_{50}s except *N*-SAR for which an indicative TD_{50} has been predicted. There is good agreement between the ranking of these *N*-NAs in the EFSA and EMA opinions for the *N*-NAs occurring in food although the approaches and methodologies used for the risk assessment of nitrosamines in the food and medicines sectors differ in some aspects. In order to indicate this point, the text in the section 1.3.7 on previous assessments has been modified.

			detected in pharmaceuticals are structural derivatives of the active pharmaceutical ingredient (API). • EFSA use the margin of exposure (MOE) as a tool to consider possible safety concerns and in their most conservative approach applied a carcinogenic potency equal to the most potent nitrosamine to the other nitrosamines. The point of departure (NDEA BMDL 10) used as the reference point for the all the other nitrosamines was also different to the point of departure generally for derivation of acceptable intakes for medicines. The approach for medicines follows the principles outlined in the ICH M7 guideline where an acceptable intake is established specifically for each nitrosamine. The point of departure for establishing the AI for nitrosamine impurities in medicinal products is usually the TD50 and this is converted to a specification limit using the maximum daily dose of the medicinal product. This is aligned with the approaches used internationally by medicines regulators. • While EFSA used the cumulative quantity of all nitrosamines potentially present in food to characterise the risk, the approach for medicines is product-specific and does not consider contamination with nitrosamines arising from other sources e.g. other medicines arising from polypharmacy. Consequently, it is recommended to include a statement in the assessment report that the conclusions and recommendations do not apply to medicines.	
	63	5852: Recommendations	The recommendations to fill data gaps, characterize metabolic activation pathways and determine relative mutagenic potencies of nitrosamines align closely with the EMA-funded studies already being performed by a consortium led by the Fraunhofer institute and expected to complete at the end of 2023. https://www.item.fraunhofer.de/en/press-and-media/press-releases/mutagenicity-of-nitrosamines.html	Thank you for informing about ongoing research activities. Once new scientific evidence justifying a revision is available EFSA will certainly consider it.
CLITRAVI	64	General comment	With the method we are using (MRM (GC-QQQ-MS)), we are only detecting NDBA and NDPheA in samples of commercial cured products (sausages and hams), but for the moment we have analysed very few samples, because we were evaluating the sensitivity of the technique. This week we will start processing a larger number of samples. In the samples we have analysed, all the other NAs	Thank you for this information. EFSA and EC would appreciate to receive the results as soon as they are available.

			(except NDMA, which cannot be detected with this technique and must be analysed in another way), are below the detection limits of the technique. But as I say, these results have to be taken with great caution for the moment, because we still have to analyse many samples. Another part of the work we are doing, and which EFSA recommended in its 2017 re-evaluation, is to try to correlate the amounts of NAs detected in the sausages with the amounts of nitrifiers added. This month we have produced one-month cured sausages (salchichon) with known amounts of nitrites (0, 75 and 150 mg/kg) and we have to wait for the end of maturation to do the analysis. The first results will be available next month. We will also repeat the same experiment to have at least one duplicate. We are sorry for not being able to have more conclusive results, but this part of the project has been delayed until we have been able to improve the method of analysis of the NAs.	
	65	lines 796 to 810	CLITRAVI Would like to know If the bibliography list includes references about: how ascorbate/eryhtorbate prevents the production on nitrosamine in meat products, the importance of this fact is underestimated in the document (lines 808 to 810): Lines 796-807: Another important chemical aspect in the mechanisms of *N*-NA formation is the possible inhibition obtained by adding some compounds to the product formulation. The possible use of ascorbates and erythorbates for NDMA mitigation in meat was first proposed by Fiddler et al. (1973). This effect was confirmed in many subsequent studies (Mirvish, 1981; Tannenbaum et al., 1991), becoming a treatment that has been required by the USDA since the early 1980s (McCutcheon, 1984). The use of these compounds accelerates the chemical conversion of nitrite to nitric oxide, inhibiting the nitrosamine formation (Archer et al., 1975; FAO/WHO, 2019). Hermmann et al. (2015b) reported up to 75% reduction of NHPRO, NPRO, NPIP and NTCA in meat products with added erythorbic acid. In the same study, the authors verified a slight decrease of NPIP, NSAR and NPYR levels in meat products supplemented with haem (e.g. ~28% for NPIP), due to increased	Three additional relevant references have been added in the text. Please see also answers to comment 9.

			competition for the nitrosating agents because more nitric oxide was bound to haem. Lines 808-810: Moreover, the authors specified that the addition of Fe(III) (added both as haem iron in the form of myoglobin from equine heart and free iron as Fe2(SO4)3 • H2O) annulled the inhibiting effect of erythorbic acid, especially for NTCA and NMTCA.	