



## **EFSA Panel on Biological Hazards (BIOHAZ); Scientific Opinion on Scientific Opinion on risk based control of biogenic amine formation in fermented foods**

### **EFSA Publication**

*Link to article, DOI:*  
[10.2903/j.efsa.2011.2393](https://doi.org/10.2903/j.efsa.2011.2393)

*Publication date:*  
2011

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

[Link back to DTU Orbit](#)

*Citation (APA):*  
EFSA Publication (2011). *EFSA Panel on Biological Hazards (BIOHAZ); Scientific Opinion on Scientific Opinion on risk based control of biogenic amine formation in fermented foods*. European Food Safety Authority. the EFSA Journal Vol. 9(11) No. 2393 <https://doi.org/10.2903/j.efsa.2011.2393>

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## SCIENTIFIC OPINION

### Scientific Opinion on risk based control of biogenic amine formation in fermented foods<sup>1</sup>

EFSA Panel on Biological Hazards (BIOHAZ)<sup>2, 3</sup>

European Food Safety Authority (EFSA), Parma, Italy

#### ABSTRACT

A qualitative risk assessment of biogenic amines (BA) in fermented foods was conducted, using data from the scientific literature, as well as from European Union-related surveys, reports and consumption data. Histamine and tyramine are considered as the most toxic and food safety relevant, and fermented foods are of particular BA concern due to associated intensive microbial activity and potential for BA formation. Based on mean content in foods and consumer exposure data, fermented food categories were ranked in respect to histamine and tyramine, but presently available information was insufficient to conduct quantitative risk assessment of BA, individually and in combination(s). Regarding BA risk mitigation options, particularly relevant are hygienic measures to minimize the occurrence of BA-producing microorganisms in raw material, additional microbial controls and use of BA-nonproducing starter cultures. Based on limited published information, no adverse health effects were observed after exposure to following BA levels in food (per person per meal): a) 50 mg histamine for healthy individuals, but below detectable limits for those with histamine intolerance; b) 600 mg tyramine for healthy individuals not taking monoamino oxidase inhibitor (MAOI) drugs, but 50 mg for those taking third generation MAOI drugs or 6 mg for those taking classical MAOI drugs; and c) for putrescine and cadaverine, the information was insufficient in that respect. Presently, only high-performance liquid chromatography (HPLC)-based methods enable simultaneous and high sensitivity quantification of all BA in foods, hence are best suited for monitoring and control purposes. Monitoring of BA concentrations in fermented foods during the production process and along the food chain would be beneficial for controls and further knowledge. Further research on BA in fermented foods is needed; particularly on toxicity and associated concentrations, production process-based control measures, further process hygiene and/or food safety criteria development, and validation of analysis methods.

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#### KEY WORDS

Biogenic amines, histamine, tyramine, fermented food, formation, criteria

<sup>1</sup> On request from EFSA, Question No EFSA-Q-2009-00829, adopted on 21 September 2011.

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<sup>3</sup> Acknowledgement: The Panel wishes to thank the members of the Working Group on risk based control of biogenic amines formation in fermented foods: Sara Bover Cid, Sava Buncic, James McLauchlin, Miguel Prieto Maradona, Elke Rauscher-Gabernig and Giuseppe Spano for the preparatory work on this scientific opinion and EFSA staff: Renata Leuschner, Stefano Cappé and Aglika Hristova for the support provided to this scientific opinion.

## SUMMARY

The consumption of food containing higher amounts of toxic biogenic amine(s) may cause food intoxication and indicates the need for a better hygiene process and other controls. Member States informed the EFSA that findings of certain levels of toxic biogenic amines (BA) in fermented food could be of concern and reported a recent increase of biogenic amines content in some fermented foods. The BIOHAZ Panel was asked to address several terms of reference (TORs) that were focused on: analysis and evaluation of the current knowledge on BAs in the context of modern food chain with particular focus on fermented foods, the BA risks for consumers' health, BA risk mitigation options, the BA monitoring including testing methodologies; as well as on identification of areas where further research and data collection are needed.

The BIOHAZ Panel conducted a qualitative risk assessment of BA in fermented foods using data from the scientific literature, as well as from relevant surveys, reports and consumption data from the MS. Based on this assessment, the BIOHAZ Panel concluded that the present knowledge and data on toxicity of biogenic amines (BA) individually and in combination(s) are limited: nevertheless, histamine and tyramine are considered as most toxic and particularly relevant for food safety. The main pre-requisites for the presence of BA in foods include: availability of free amino acids, the presence of microorganisms producing BA enzymes (mainly from raw materials and/or added starter cultures), and conditions allowing their growth (particularly temperature, pH), as well as conditions affecting the enzyme production and activity (particularly low pH). Fermentation of foods provides the conditions indicated above allowing intensive microbial activity and therefore has the potential for BA formation. Storage and distribution conditions (in particular temperature) for fermented foods are variable in practice and may be relevant for BA accumulation.

Among groups of foods and within each group indicated below there is variability in amounts of BA found. Data are inadequate for differentiating individual products within each group. Based on the mean content of the most toxic BA (histamine and tyramine), the food safety relevance of the considered food categories can be ranked in following decreasing order - for **histamine**: 'dried anchovies', 'fish sauce', 'fermented vegetables', 'cheese', 'other fish and fish products' and 'fermented sausages'; and for **tyramine**: 'fermented sausages', 'fish sauce', 'cheese', 'fermented fish' and 'fermented vegetables'. Based on the consumer exposure to the most toxic BA, the food safety relevance of the considered food categories can be ranked in following decreasing order - for **histamine**: 'other fish and fish products', 'fermented sausages', 'cheese', 'fish sauces' and 'fermented vegetables'; and for **tyramine**: 'beer', 'cheese', 'fermented sausages', 'fermented fish meat' and 'preserved meat'. For quantitative risk assessment, further information and data on BA (individually and in combination(s)) including toxicity, concentration and consumption of fermented foods are required.

BA accumulation in fermented foods is a complex process affected by multiple factors and their interactions, the combinations of which are numerous, variable and product-specific. Hence, risk mitigation options, which are based on controlling those factors/interactions, could not be considered and ranked individually. Rather, they were considered at general principles level. Minimizing the occurrence of BA-producing microorganisms can be achieved through ensuring the good hygienic status of the raw material and, where possible, additional microbial controls. Microorganisms intended to be used as starter cultures in any fermented food should be confirmed as not producing BA and able to outgrow autochthonous microbiota under conditions of production and storage. All aspects of fermented food processing (including ingredients, fermentation and ripening regimes), distribution and storage should be adjusted and balanced in each particular product to avoid/minimize potential enhancing effects on BA formation and to enable dominance of starter culture(s) where used.

Estimating safe levels of the total amounts of BA ingested is the key issue to understand health effects to consumers. Consumption data and the exposure assessment were used by the Panel to define the concentrations in food that would be allowable, however these will vary between individuals, regions

and countries. Therefore, for the purpose of this document, the focus was on total amounts of BA ingested in relation to estimated threshold levels for BA. For histamine, currently, available information needed for establishing NOAEL and ARfD in humans is based only on limited number of healthy and sensitive individuals. Based on limited published information, no adverse health effects have been observed in healthy volunteers exposed to a level of 25 to 50 mg of histamine per person per meal. This level may be occasionally exceeded by consumption of one or more food items containing high amounts of histamine during the same meal. In patients with histamine intolerance, even small amounts of histamine in ingested food may cause adverse health effects, so only levels below detectable limits can be considered as safe. For tyramine, there is currently insufficient information related to establishing a NOAEL in humans. Based on limited published information, no adverse health effects have been observed in healthy individuals not taking monoamino oxidase inhibitor (MAOI) drugs exposed to a level of 600 mg of tyramine per person per meal. This level would not be exceeded even by a combined high intake of the five main food sources of tyramine during the same meal. In individuals taking third generation MAOI drugs, no adverse health effects have been observed after exposure to a level of 50 mg of tyramine per person per meal. High consumption of some fermented foods (beer, cheese, fermented sausages and fermented fish meat) can lead to tyramine exposure exceeding this level. For individuals taking classical MAOI drugs, no adverse health effects have been observed after exposure to a level of 6 mg of tyramine per person per meal. This would be easily exceeded by the consumption of fermented food. For putrescine and cadaverine, presently available information is insufficient to identify concentrations that directly cause acute adverse health effects and/or potentiate the toxic effects of histamine and other biogenic amines.

Presently, high-performance liquid chromatography (HPLC)-based methods are the only methods which reliably and with high sensitivity can simultaneously quantify concentrations of all BA in fermented food, therefore, are most suitable for analysis of fermented foods. Currently, there is insufficient information in order to recommend detailed monitoring schemes and methods. Monitoring of BA concentrations in fermented food during the production process could be used as one of the parameters for the process hygiene assessment. Monitoring of raw materials and products at multiple points along the food chain is necessary to evaluate the relevance of various factors contributing to BA formation and accumulation in fermented foods.

The BIOHAZ Panel recommended that further research is needed on: the toxicity and associated concentrations of histamine and tyramine in different foods, as well as related potentiating effects of putrescine and cadaverine, in particular concerning food involved in outbreaks and sporadic cases; the consumption data of fermented foods, especially cheese; the production process-based control measures for BA in fermented food including monitoring and verification aspects and the development of challenge tests; the evaluation of the need for and, if/where necessary, development of process hygiene criteria for histamine and tyramine in fermented foods, as well as food safety criteria for histamine in fermented foods other than fish. Also, validation of methods for BA analysis is recommended for all relevant food types including standardisation and harmonisation of procedures, external quality assessment and availability of certified reference materials.

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## BACKGROUND AS PROVIDED BY EFSA

Member States informed the EFSA Advisory Forum in June 2009<sup>4</sup> that findings of certain levels of toxic biogenic amines in fermented food could be of concern. The same Member States reported a recent increase of biogenic amines content in some fermented foods at the EFSA Network meeting on Microbiological Risk Assessment held also in early June 2009<sup>5</sup>.

The consumption of food containing higher amounts of toxic biogenic amine(s) may cause food intoxication with symptoms including flushing, headaches, nausea, cardiac palpitations, and increased or decreased blood pressure; in extreme cases the intoxication may have fatal outcome.

Biogenic amines are non-volatile low-molecular weight nitrogenous organic bases, derived through decarboxylation of corresponding amino acids. They can be both formed and degraded as a result of normal metabolic activities in humans, animals, plants and microorganisms. The responsible enzymes, amino acid-decarboxylases, are widely present in spoilage and other microorganisms e.g. in naturally occurring and/or artificially added lactic acid bacteria involved in food fermentation. Biogenic amines are thermo-stable and are not inactivated by heat treatments used in food processing and preparation. Presence of higher concentrations of toxic biogenic amines in food is undesirable and indicates the need for a better hygiene process and control.

Formation of biogenic amines in all foods of animal origin having high protein contents, as well in foods of plant origin, has been reported. It can occur as a result of activities of spoilage microflora and/or intentionally added microorganisms. Therefore, microbial starter cultures should be selected and controlled so that, during the fermentation process, they do not produce biogenic amines of concern. While histamine has received much attention (also tyramine although to a lesser extent) due to higher toxicity, there are numerous reports in the literature concerning other biogenic amines such as putrescine, phenylethylamine and cadaverine; the latter three at least can potentiate the negative effects of the former two. Normally, amine-oxidising enzymes catabolise and inactivate ingested biogenic amines; however, activity of these enzymes can be suppressed by some factors. The toxic effects of biogenic amines from foods have been observed particularly in individuals having dysfunctional biogenic amines-degrading mechanisms either naturally or due to intake of alcohol or certain medications.

As indicated above, amongst the biogenic amines, histamine has attracted particular attention, as it has been implicated as the causative agent in a number of outbreaks of food poisoning. Histamine poisoning is a foodborne intoxication following the ingestion of foods containing excessive amounts of a microbial metabolite - histamine. Although it is commonly associated with the consumption of scombroid-type fish, other foods such as cheese and wine have also been implicated in outbreaks of histamine poisoning. Nevertheless, it should be noted that some concern exists also with respect to potentially toxic effects of excessive amounts of tyramine that can be present in some foods such as fermented sausages and cheeses.

Formation of biogenic amines can occur during food processing and storage as a result of bacterial activities. Consequently, higher amounts of certain amines may be found in foods as a consequence of the use of poor quality raw materials, microbial contamination and inappropriate conditions during food processing, and microbial contamination and inadequate conditions during storage. There is evidence that as the hygienic quality of the product decreases, the biogenic amine content increases. Therefore, a number of published studies explored possibilities of using the amine concentrations as a parameter of process hygiene and food spoilage/quality.

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<sup>4</sup> Minutes of the thirty second meeting of the Advisory Forum, 24-25 June 2009, p. 8

<sup>5</sup> Minutes of the 3<sup>rd</sup> meeting of the EFSA Network on Microbiological Risk Assessment, June 2009

Commission Regulation (EC) 2073/2005<sup>6</sup> lays down food safety criteria for histamine in fishery products from fish species associated with a high amount of histidine between 100 mg/kg (m) and 200 mg/kg (M) (n=9, c=2) and for fishery products which have undergone enzyme maturation treatment in brine, manufactured from fish species associated with a high amount of histidine between 200 mg/kg (m) and 400 mg/kg (M) (n=9, c=2).

Regulation (EC) No 853/2004<sup>7</sup> provides for fishery products a possibility to lay down freshness criteria and limits with regard to histamine and places the responsibility on food business operators to ensure that the limits with regard to histamine are not exceeded in the context of health standards for these products.

### **TERMS OF REFERENCE (ToR) AS PROVIDED BY EFSA**

- ToR1 Carry out a review of the available published scientific information on biogenic amines in foods with regards to production, processing, transport, storage/retail (i.e. during the shelf-life of the products) until consumption; including on consumer exposure and potential health implications.
- ToR2 Carry out a risk profiling of relevant fermented foods regarding biogenic amine formation from production to consumption (within their 'use-by' date). Include data from additional sources where applicable. The profiling should also result in identification of data needs to support a quantitative risk assessment.
- ToR3 Identify and rank possible risk mitigation options and their anticipated impact to prevent or limit biogenic amines formation in fermented foods.
- ToR4 Characterise concentration levels of biogenic amine in relevant fermented foods that are not associated with adverse health effects of defined consumer groups including susceptible consumers.
- ToR5 Give advice regarding the analytical method to measure biogenic amine concentrations in fermented foods.
- ToR6 Recommend the monitoring methods in fermented foods that are most relevant from the public health point of view. These recommendations may refer to, among other aspects, the fermented food categories, the stages during food production until consumption within the shelf-life of a product to be sampled, as well as the type of sample to be collected.

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<sup>6</sup> OJ L 338, 22.12.2005, p. 11,12. Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs amended by Regulation (EC) No 1441/2007 (OJ L 322, 7.12.2007, p.17,18)

<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32005R2073:en:NOT>

<sup>7</sup> OJ L 139, 30.4.2004, p. 30, 68, Corrigendum to Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April laying down specific hygiene rules for food of animal origin



## ASSESSMENT

### 1. Introduction

The primary relevance of biogenic amines (BA) is that intake of foods or beverages containing high concentrations of biogenic amines can present a health hazard through the direct toxic effect of these compounds and their interaction with some medical treatments; but also they may have a role as indicators of quality and/or acceptability in some foods (Shalaby, 1996; Ruiz-Capillas and Jiménez-Colmenero, 2004). As food safety is the main focus of this document, attention will primarily be on the former aspect. As neither the most toxic (histamine and tyramine) nor other BAs are significantly affected by normal cooking or other processing of food or beverages makes food safety assurance in respect to BA more challenging. Presently, the main BA control strategies are focused on prevention of BA formation in foods at all relevant points in the food chain.

However, despite extensive research on food safety aspects of BA including risk mitigation options for most relevant foods, some related aspects are still poorly understood. Also, risk assessment of consumer's health-relevant concentrations of biogenic amines in foods (especially fermented foods) has been difficult because published information on toxic effects, dose-response and the actual concentrations in foods is quite limited. Furthermore, the conditions in food production-storage-retailing-preparation chain have changed including modifications in some traditional practices and the use of new/emerging technologies, which may produce differences in the formation and/or concentrations of different compounds including BAs in related food types. Furthermore, global food trade and consumers' eating habits – both food safety relevant factors - are changing. Therefore, there is a need to periodically re-visit the BA-related food safety issues and consider any new related knowledge, data and trends which may enable further improvements of BA risk reduction strategies.

Therefore, the main scope of this document is, briefly, to analyse and evaluate: the current knowledge on BAs in the context of modern food chain with particular focus on fermented foods, the BA risks for consumers' health, BA risk mitigation options, the BA monitoring including testing methodologies; as well as to identify areas where further research and data collection are needed.

### 2. Hazard Identification

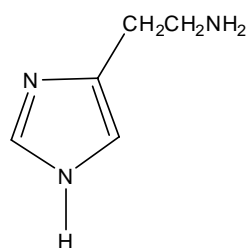
#### 2.1. Background

Biogenic amines (BA) are organic, basic, nitrogenous compounds of low molecular weight, mainly formed by the decarboxylation of amino acids and with biological activity. According to their chemical structure, they can be classified as heterocyclic (histamine and tryptamine), aliphatic (putrescine and cadaverine) or aromatic (tyramine and phenylethylamine) (Figure 1). According to their number of amine groups they can be divided into monoamines (tyramine and phenylethylamine) and diamines (histamine, putrescine and cadaverine). BAs are naturally occurring in animals and humans. They are involved in natural biological processes such as synaptic transmission, blood pressure control, allergic response and cellular growth control. Nonetheless, BA may be hazardous to human health if their levels in foods or beverages reach a critical threshold (Ladero et al., 2010a). The main source of exogenous amines is dietary, through the uptake of foods or beverages containing high concentrations of these compounds (Shalaby, 1996; Silla Santos, 1996; Premont et al., 2001). Foods likely to contain high levels of biogenic amines include fish, fish products and fermented foodstuffs (meat, dairy, vegetables, beers and wines). The most important BAs found in food are histamine, tyramine, putrescine, cadaverine and phenylethylamine, which are products of the decarboxylation of histidine, tyrosine, ornithine, lysine and phenylalanine, respectively. Putrescine can also be formed through deimination of agmatine. Microorganisms possessing the enzyme decarboxylases, which convert amino acids to amines, are responsible for the formation of biogenic amines in foods (Beutling, 1996).

BA production in foods requires the availability of precursors (i.e. amino acids), the presence of bacteria synthesising amino acid decarboxylases, and favourable conditions for their growth and decarboxylating activity (Ten Brink et al., 1990; Stratton et al., 1991). The amount and type of biogenic amines formed in foods is strongly influenced by the intrinsic food characteristics including pH, water activity, composition, microbiota and by extrinsic parameters such as storage time and temperature, which allow bacterial growth during food processing and storage.

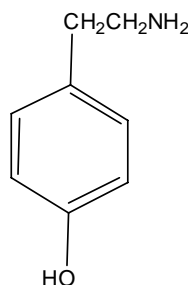
The biogenic amine content (particularly) of some foods has been widely studied because of their potential toxicity. Histamine has been implicated as the causative agent in outbreaks of food poisoning where intoxication results from the ingestion of foods containing excessive amounts of histamine. Although commonly associated with the consumption of scombroid-type fish, other foods such as cheese have also been associated with outbreaks of histamine poisoning. Tyramine and phenylethylamine have been identified as the initiators of hypertension during treatment with monoamino oxidase inhibitor (MAOI) drugs and of dietary-induced migraine in susceptible individuals.

### Heterocyclic amines

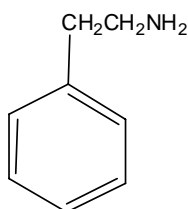


Histamine

### Aromatic amines

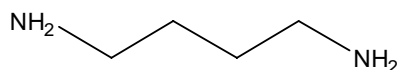


Tyramine

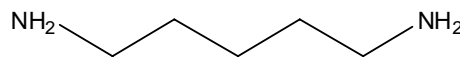


2-Phenylethylamine

### Aliphatic amines



Putrescine



Cadaverine

**Figure 1:** Structure of histamine, tyramine, 2-phenylethylamine, putrescine and cadaverine

## 2.2. Microorganisms with amino acid decarboxylase activities

Biogenic amines in food are mainly formed by bacterial decarboxylation of the corresponding amino acids through substrate-specific decarboxylase enzymes (Figure 2). Biodegradative decarboxylases do

not show a strict specificity towards the substrates and when the primary amino acid substrate is absent, structurally similar amino acids may be decarboxylated to form other biogenic amines. For instance, ornithine-decarboxylases generate cadaverine from lysine (Bardocz, 1995), arginine-decarboxylases decarboxylate ornithine at slow rate (Tabor and Tabor, 1985), and tyrosine-decarboxylases form phenylethylamine from phenylalanine (Joosten, 1988). The lack of substrate specificity of amino acid decarboxylases provides a mechanism for 'emergency' pH homeostasis since this shows some independence from the available extracellular amino acids (Foster and Hall, 1991, Park et al., 1996).

The production of biogenic amines has been associated with some groups of microorganisms. For example, putrescine and cadaverine production is frequently found in enterobacteria, and tyramine production is reported in the majority of enterococci. Within microbial groups, the capacity to produce biogenic amines is, however, a strain-specific characteristic, more widely distributed among certain genera or species, suggesting that horizontal gene transfer may account for their dissemination between strains (Coton and Coton, 2009; Lucas et al., 2005; Marcobal et al., 2006a). Moreover, great variation has been reported in the type and quantity of biogenic amines produced between different strains of the same species. Some strains are even able to simultaneously produce more than one amine simultaneously, either due to the presence of different decarboxylases or to the action of a single enzyme which decarboxylates different amino acids (Bover-Cid and Holzapfel, 1999).

### 2.2.1. Decarboxylases

Decarboxylases belong to the pyridoxal-phosphate-dependent enzyme group, whose members use pyridoxal-5'-phosphate (PLP) as a coenzyme. Histidine decarboxylases fall into two different classes: those from eukaryotic cells and Gram-negative bacteria, which require pyridoxal phosphate as a cofactor (Guirard and Snel, 1987), and those from Gram-positive bacteria, which use a covalently bound pyruvoyl moiety as the prosthetic group (Recsei and Snell, 1984).

Tyrosine decarboxylase (TDC) converts tyrosine to tyramine and also catalyses L-dopa decarboxylation. TDC enzymes are usually also able to use phenylalanine as a substrate to produce phenylethylamine; this enzyme is therefore also responsible for the phenylethylamine content detected in some food products. This dual activity has recently been confirmed with the expression of TDC enzyme from *Enterococcus faecium* RM58 in *E. coli*, which showed phenylalanine and tyrosine decarboxylase activities (Marcobal et al., 2004a; Marcobal et al., 2006b). TDC in *Lactobacillus brevis* is highly specific for tyrosine (Moreno-Arribas and Lonvaud-Funel, 1999).

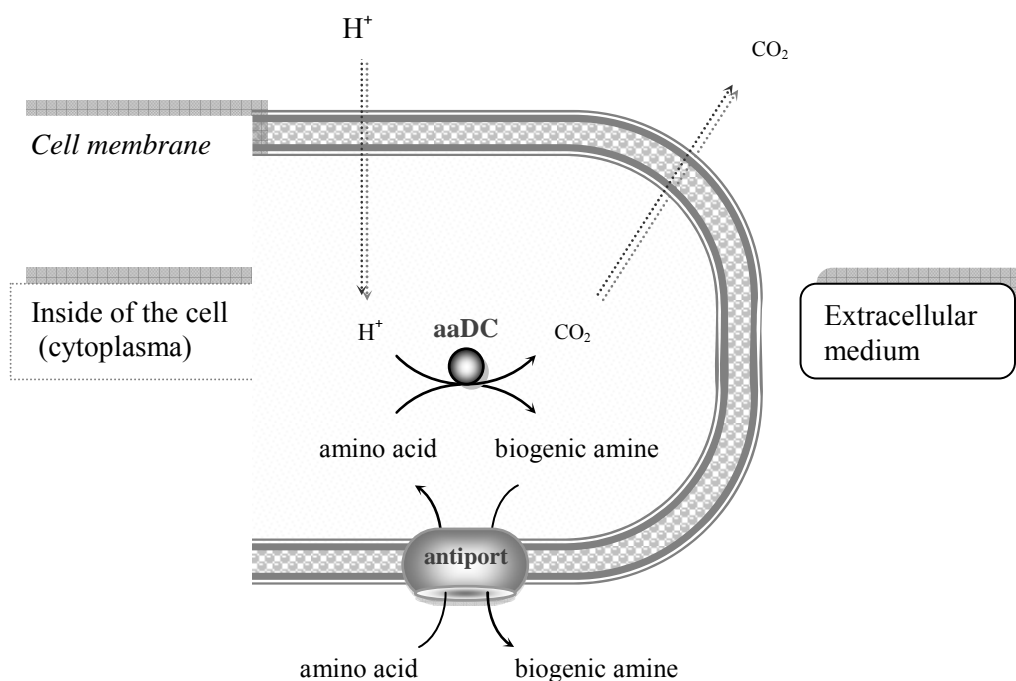
Cadaverine can be formed from lysine by the lysine decarboxylase activity. Cadaverine production has been generally associated either with Gram-negative bacteria, though some Gram-positive bacteria have also been described to be able to produce cadaverine in a lysine-rich medium (Bover-Cid and Holzapfel, 1999).

Putrescine can be formed from ornithine by ornithine decarboxylase. Alternatively, arginine can be converted to agmatine by arginine decarboxylase and agmatine can be converted to putrescine by the agmatine deiminase system which is formed by three enzymes: agmatine deiminase, putrescine carbamoyltransferase and carbamate kinase (Arena and Manca de Nadra, 2001).

### 2.2.2. The transporter proteins

After amino acid decarboxylation, the second component in the aminogenesis process is an inner membrane antiporter used to deliver the amino acid substrate into the cell and to remove (excrete) the decarboxylated product from the cytoplasm. In the amino acids/amines antiporter, the uptake of substrate and excretion of products are coupled events (Figure 2).

The presence of a Histidine/Histamine antiporter (HdcP) was reported in *Lactobacillus buchneri* ST2A (Molenaar et al., 1993). In whole cells of this bacterium, the coupled reactions of histidine decarboxylation and histidine/histamine exchange generated a transmembrane pH gradient (inside alkaline) and an electrical potential (inside negative), i.e., a proton motive force (PMF) (secondary metabolic energy generation). Similar mechanisms for the other amino acid/biogenic amine have also been characterised in other microorganisms, for instance the tyrosine/tyramine transport mechanism on *Lactobacillus brevis* (Wolken et al., 2006) or the putrescine and cadaverine transport proteins (PotE and CadB respectively) have been well characterised in *Escherichia coli* (Soksawatmaekhin et al., 2004).



**Figure 2:** Biogenic amine biosynthesis pathways in bacteria. Amino acid decarboxylase (aaDC). The membrane antiporter protein delivers the amino acid substrate into the cell and removes (excretes) the decarboxylated product from the cytoplasm. (Adapted from Bover-Cid, 2000a)

### 2.2.3. Histamine producing microorganisms

The ability to produce histamine has been found in both Gram-negative and in Gram-positive bacteria.

Many Gram-negative bacteria which commonly contaminate food are able to produce histamine. The strongest histamine producers *Hafnia alvei*, *Morganella morganii*, *Klebsiella pneumonia* and, more recently, *Morganella psychrotolerans*, *Photobacterium phosphoreum*, *Photobacterium psychrotolerans* and have been isolated from fish incriminated of scombroid poisoning incidents (Özogul and Özogul, 2006; Kanki et al., 2004; Emborg et al., 2006; Dalgaard et al., 2008).

In fermented food, for example strains of *Oenococcus oeni*, *Pediococcus parvalus*, *Pediococcus damnosus*, *Tetragenococcus* species, *Leuconostoc* species, *Lactobacillus saerimneri* 30a, *Lactobacillus hilgardii*, *Lactobacillus buchnerii* and *Lactobacillus curvatus*, are known to produce histamine (Vanderslice et al., 1986; Spano et al., 2010; Beutling, 1996; Kimura et al., 2001; Lucas et al., 2005). Histamine producing *Lactobacillus parabuchneri* or *Lactobacillus rossiae* strains have been found as contaminating microbiota in starter culture preparations used in winemaking (Costantini et al., 2009).

In the recent years, histamine formation, or the occurrence of the *hdc* gene, have been occasionally described for some specific species previously unrecognised as being able to produce biogenic amines, such as *Streptococcus salivarius* subsp. *thermophilus* (Calles-Enriquez et al., 2010) and *Lactobacillus sakei* (Coton and Coton, 2005). However, the proportion of decarboxylase-positive strains is low and their histaminogenic potential is weak or not proven in food.

Kanki et al. (2007) suggested that the enzymatic activity of histidine decarboxylase was responsible for histamine formation and its action could continue even after bacterial autolysis. This was confirmed in experiments using recombinant histidine decarboxylase (HDCs) of the histamine-producing bacteria *Photobacterium phosphoreum*, *P. damsela*, *R. planticola*, and *M. morganii* in which the bacteria themselves were absent (Kanki et al., 2007).

#### 2.2.4. Tyramine and phenylethylamine producing microorganisms

The main tyramine producers in cheese and fermented sausages are Gram-positive bacteria within the genera *Enterococcus* (e.g. *Enterococcus faecalis* and *Enterococcus faecium*), *Lactobacillus* (e.g. *Lactobacillus curvatus* and *L. brevis*) (Bover-Cid et al., 2000b,c), *Leuconostoc* and *Lactococcus* (Fernandez et al., 2004; Fernandez et al., 2007a,b) and *Carnobacterium* spp. (Masson et al., 1999). *Staphylococcus* may also have a role in the production of tyramine (Ansorena et al., 2002; Martin et al., 2006; de las Rivas et al., 2008; Latorre-Moratalla et al., 2010a,b). In fermented beverages, *L. brevis*, *L. hilgardii*, *Lactobacillus plantarum* and *Leuconostoc* species have been described as tyramine producers (Coton et al., 2010a).

Phenylethylamine production has been described during food fermentations and has been suggested to be formed as a result of the activity of tyrosine decarboxylating bacteria towards this structurally related amino acid. Usually, phenylethylamine production is associated with tyramine production as demonstrated for *Enterococcus*, *Lactobacillus curvatus*, *Staphylococcus*, (Bover-Cid et al., 2001a,b,c; Bover-Cid et al., 2003; Moreno-Arribas and Lonvaud-Funel 2001; Suzzi and Gardini 2003). However, purified tyrosine decarboxylase from *L. brevis* does not demonstrate the ability to decarboxylate phenylalanine; instead it is tyrosine specific (Moreno-Arribas and Lonvaud-Funel, 2001).

Interestingly, some strains of *S. carnosus* from fermented sausages have been reported to produce considerable amounts of phenylethylamine without producing tyramine (Ansorena et al., 2002; de las Rivas et al., 2008; Latorre-Moratalla et al., 2009).

#### 2.2.5. Putrescine and cadaverine producing microorganisms

Putrescine and cadaverine production has mainly been related to Gram-negative bacteria, especially in the families *Enterobacteriaceae*, *Pseudomonadaceae* and *Shewanellaceae*, generally associated with spoilage (Lopez-Caballero et al., 2001). Enterobacteria genera *Citrobacter*, *Klebsiella*, *Escherichia*, *Proteus*, *Salmonella* and *Shigella* are associated with production of considerable amounts of putrescine and cadaverine in food (Paleologos et al., 2004; Kim et al., 2009; Rezaei et al., 2007; Chytiri et al., 2004). The ornithine and lysine decarboxylase phenotypes within *Enterobacteriaceae* isolates have been traditionally used for taxonomic purposes (Moeller, 1954).

Putrescine is one of the most common BA found in fermented products. Lactic acid bacteria, lactobacilli mainly, and staphylococci have also been reported to be able to produce putrescine and/or cadaverine (Arena et al., 2001; Beneduce et al., 2010; Coton et al., 2010b).

Putrescine has been described to be produced mainly by the dominant malolactic fermentation (MLF) species in wine, *Oenococcus oeni*, although strains belong to *L. hilgardii* and *L. plantarum* species are able to produce putrescine during wine fermentation (Nannelli et al., 2008; Arena et al., 2001; Marcobal et al., 2004b; Lucas et al., 2007).



### 2.2.6. Physiological role of decarboxylases in microorganisms

In microorganisms, two types of amino acid-decarboxylase enzymes have been described having different physiological roles: biosynthetic (constitutive) mainly associated with microbial growth (Kamio et al., 1986; Kamio and Nakamura, 1987) and biodegradative (inducible by a number of environmental factors) that fulfil a number of physiological roles depending on the microorganisms (Tabor and Tabor, 1985; Applebaum et al., 1977). The constitutive biosynthetic decarboxylases are present in considerably less quantities than that of the induced biodegradative forms (Suzuki et al., 1991) whereby the latter are responsible for the biogenic amine accumulation in foods.

In prokaryotic cells, the physiological role of BA synthesis by biodegradative decarboxylases mainly appears to be related to defence mechanisms used by bacteria to withstand acidic environments (Lee et al., 2007, Rhee et al., 2002). Decarboxylation increases survival under acidic stress conditions via the consumption of protons and the excretion of amines and CO<sub>2</sub>, helping to restore the internal pH (Figure 2). BA production may also offer a way of obtaining energy, since the electrogenic amino acid/amine antiport can lead to generation of proton motive force (Molenaar et al., 1993). This function is particularly important to microorganisms lacking a respiratory chain for generating high yields of ATP (Vido et al., 2004).

A role of amino acid-decarboxylases in the pathogenesis of certain bacteria is to contribute to survival in the acid contents of the stomach and food containing acidic materials. Also, some reports suggest that decarboxylases may prevent the acidification of the macrophage/phagosome after invasion of macrophage cells (Park et al., 1996). Production of tyramine may even contribute to the survival and colonisation mechanism of *Enterococcus durans* in the human colon (de Palencia et al., 2011). As this mechanism could also apply to other biogenic amines and other bacteria, this hypothesis about the production of amines (histamine and tyramine) as a protective mechanism against acidic environment was originally suggested for Gram-negative intestinal bacteria (Hanke and Koessler, 1924).

Some studies suggest new and interesting hypotheses on the physiological role of amines in microorganisms (Tkachenko et al., 2001). In *Escherichia coli*, the expression of *oxyR*, the gene that protects *E. coli* against oxidative stress, was enhanced by physiological concentrations of the BA putrescine. Moreover, putrescine was shown to produce a protective effect if the DNA is damaged by reactive oxygen species (Tkachenko et al., 2001). Putrescine may be involved in osmotic stress tolerance in *E. coli* (Schiller et al., 2000). Therefore, bacteria which possess amino acid decarboxylase activity could overcome or reduce the effects of factors that induce stress responses in the cell, such as oxygen and NaCl, with the production of putrescine.

Since histamine is a potent vasodilator, it may alter the physiology of the host to the advantage of bacteria, for instance resulting in transfer of essential nutrients from host to bacteria via secretion into the gut lumen as a result of capillary dilation (Recsei and Snell, 1984; Poelje and Snell, 1990). Those bacteria capable to produce histamine may cause tissue damage that, in turn, facilitates the colonization and invasion of the host (Barancin et al., 1998). Similarly, the activation of the tyramine biosynthetic pathway may be involved in enhancement of the adhesion between LAB and epithelium cells. These results are in agreement with those previously reported by Lyte (2004) who demonstrated an improved adhesion to the intestinal mucosa of *Escherichia coli* O157:H7 in the presence of tyramine.

Some decarboxylases may be implicated as a possible virulence factor for certain foodborne pathogens. A plasmid encoded histidine decarboxylase has been described in *Vibrio anguillarum* (Barancin et al., 1998), which may play a role in the biosynthetic pathway of its siderophore and consequently in conferring the high virulence to this bacterium (Tolmasky et al., 1995). Similarly, putrescine has been described as an essential precursor for alcaligin siderophore biosynthesis in *Bordetella* species (Brickman and Armstrong, 1996).

## 2.3. Conditions supporting the formation of BAs

### 2.3.1. Substrate availability

The availability of substrate amino acids is one of the prerequisites for BA synthesis. Proteolysis is a crucial factor, because it is directly related to availability of free amino acids that provide a substrate for BA formation. It has been reported that conditions of accelerated or enhanced proteolysis increases BA formation (Leuschner et al., 1998a; Innocent and D'Agostin, 2002; Fernández et al., 2007a; Komprda et al., 2008).

### 2.3.2. Conditions allowing growth of BA producers, enzyme production and activity

#### 2.3.2.1. Effect of temperature

The quantitative production of biogenic amines is usually reported to be temperature and time dependent (Zaman et al., 2009). Generally the amine production rate increases with the temperature. Conversely, biogenic amine accumulation is minimised at low temperatures through inhibition of microbial growth and the reduction of enzyme activity. The optimum temperature for the formation of BA by mesophilic bacteria has been reported to be between 20 to 37 °C, while production of BA decreases below 5 °C or above 40 °C. For instance, *Morganella morganii* is known to be a powerful histamine producer in seafood, though at storage temperatures above 7-10 °C (Kim et al., 2002; Lehane and Olley, 2000). *Klebsiella pneumoniae* was reported to produce cadaverine more extensively at 20°C than at 10°C, whereas *Enterobacter cloacae* was able to produce putrescine at 20°C but not at 10°C (Halász et al., 1994). In refrigerated foods (e.g. chilled fresh fish stored in ice) psychrotolerant bacteria can actively contribute to the amine accumulation at high rates even during storage below 5°C, *Photobacterium phosphoreum* and *Morganella psychrotolerans* (a psychrotolerant variant of *M. morganii*) being the most relevant bacteria (Lakshmanan et al., 2002; Emborg et al., 2005; Emborg et al., 2006; Daalgaard et al., 2006; Rezaei et al., 2007).

Prolonged storage periods, in particular at abuse temperatures make the food more susceptible to amine formation. Some BA such as tyramine, putrescine, and cadaverine can be formed during the storage of food (Bover-Cid et al., 2001c; Suzzi and Gardini, 2003; Maijala, 1993; Ferreira and Pinho, 2006; Kosson and Elkner, 2001). For this reason, low temperatures should be applied during storage to reduce proteolytic and decarboxylase activities and growth of bacteria (Hernández-Orte et al., 2008; Rezaei et al., 2007). However, the occurrence and activity of psychrotrophic bacteria can result in biogenic amines formation also in properly chilled stored fish (Middlebrooks et al., 1988; Kim et al., 2009; Bakar et al., 2010).

#### 2.3.2.2. Effect of pH

The pH level is an important factor influencing amino acid decarboxylase activity (Silla Santos, 1996). There are two pH related mechanisms acting simultaneously. One is affecting the growth by acidity which inhibits the growth of microorganisms (Maijala et al., 1993; Maijala, 1994; Masson et al., 1999; Bover-Cid et al., 2000c). The other affects the production and activity of the enzyme because in low pH environment, bacteria are more stimulated to produce decarboxylase as a part of their defence mechanisms against the acidity (Molenaar et al., 1993; Bover-Cid et al., 2006b; Fernández et al., 2007a). These opposing factors interfere with each other and the net result depends on their balance.

#### 2.3.2.3. Other factors

Sodium chloride influences the activity of amino acids decarboxylase involved in biogenic amine production. Histidine decarboxylase activity of *Staphylococcus capitis*, *Enterobacter cloacae* and



*Pantoea agglomerans* are retarded by the high concentration of salt. In contrast, sodium chloride enhanced activity of histidine decarboxylase of halotolerant *Staphylococcus* spp. isolated from salted anchovies. Hence, it can be assumed that the effect of sodium chloride either inhibiting and stimulating biogenic amines production is strain specific (Taylor and Woychik, 1982; Taylor and Speckard, 1984; Hernandez-Herrero et al., 1999; Rodriguez-Jerez et al., 1994).

Oxygen availability also appears to have a significant effect on the biosynthesis of BA. *Enterobacter cloacae* produces about half the quantity of putrescine under anaerobic as compared with aerobic conditions, and *Klebsiella pneumoniae* synthesizes significantly less cadaverine but acquires the ability to produce putrescine under anaerobic conditions (Halász et al., 1994). The redox potential of the medium also influences BA production. Conditions resulting in a reduced redox potential stimulate histamine production, and histidine decarboxylase activity seems to be inactivated or destroyed in the presence of oxygen (Karošičová and Kohajdová, 2005). In contrast, oxygen availability (aerobic or anaerobic growth) had little influence on the tyramine, phenylethylamine and putrescine production by *Lactobacillus curvatus* (Bover-Cid et al., 2006b).

Some other factors including tartaric acid and sugar concentration may play a role in some foods but information on their actual contribution and mechanisms of action are relatively limited. The presence of tartaric acids appears to increase the amount of putrescine produced by *L. hilgardii*. However, an inhibitory effect by sugars on the HDC catalytic activity has been reported (Arena et al., 2008; Lonvaud-Funel, 2001).

## **2.4. Main scenarios leading to the occurrence of BA in food**

### **2.4.1. Preformed BA in raw materials**

Some amines identified in fermented food are already found at low levels in raw materials of good hygienic quality such as putrescine in meat, fish, milk and fruits. Blood and offal can contain physiological concentrations of histamine, hence it can be found in products containing these raw materials such as blood sausages (Mariné-Font et al., 1995).

From a practical perspective, prediction of biogenic amine accumulation in foods as a function of time and temperature, as well as other relevant environmental factors, can be used to avoid conditions that result in unacceptable biogenic amine levels. This approach has already been developed for histamine accumulation in seafood, particularly associated with growth and histamine formation by *Morganella psychrotolerans* and *M. morganii* (Emborg and Dalgaard, 2008), and included within the Seafood Spoilage and Safety Predictor tool (<http://sssp.dtuaqua.dk>). This validated predictive tool can be used by the seafood industry within a HACCP program, since this provides valuable predictions to determine critical combinations of storage conditions enabling control of histaminogenesis in fish (Dalgaard et al., 2008).

### **2.4.2. Spoilage**

Spoilage microorganisms may strongly contribute to BA formation in fermented food even though food is not spoiled. Indeed, as results of their microbiological origin, BAs have been used as a criteria to evaluate the hygienic quality and freshness of certain foods, especially fish, but also meat products. For this purpose, a number of the so called Biogenic Amine Indices (BAIs) have been suggested, which may include one (e.g. Vinci and Antonelli, 2002) or multiple biogenic amines (e.g. Veciana-Nogués et al., 1997), even combination with other parameters (Jørgensen et al., 2000).

### 2.4.3. Fermentation

The main aim of lactic acid fermentation is the conversion of carbohydrates to lactic acid. Therefore the action of lactic acid bacteria is desirable for the production of fermented sausages, cheese, sour dough bread and also for malolactic fermentation of wine. However, some of the bacteria involved in fermentation can produce BAs.

There is no evidence about massive formation of biogenic amines by yeast and moulds, although they may bear constitutive amino acid decarboxylase enzymes involved in a number of physiological functions. The occurrence of considerable amounts of histamine and tyramine in yeast extracts (“Marmite”) was reported (Tabor and Tabor, 1985; Blackwell et al., 1969), although a potential bacterial contamination as the source of these toxic biogenic amines was identified. Therefore, alcoholic fermentation is not of concern for BA formation unless spoilage with lactic acid bacteria occurs.

### 2.5. Possible *in situ* formation of biogenic amines in the gut

In addition to the ingestion of BAs formed in food, there is a possibility of BA production in the human intestine due to the microbiota both from the intestine or the food (de Palencia et al., 2011). Intestinal bacteria may also be capable to degrade biogenic amines, either ingested with food or produced by other intestinal bacteria (Hanke and Koessler, 1924). The actual relevance for public health of BAs produced in the intestine is presently unclear and not proven.

## 3. Hazard characterisation

### 3.1. Histamine

#### 3.1.1. Synthesis and metabolism in humans

Histamine is synthesized by the pyridoxal phosphate containing L-histidine decarboxylase from the amino acid histidine in mast cells, basophils, platelets, histaminergic neurons, and enterochromaffine cells (Maintz and Novak, 2007). It can be metabolised by oxidative deamination by diamino oxidase (DAO) and by ring methylation by histamine-N-methyltransferase (HNMT) depending on its localization (Jarisch, 2004). Monoamine oxidase (MAO) is also involved in the HNMT products pathway. DAO has been shown to be a secretory protein predominantly synthesized in renal proximal tubular and intestinal epithelial cells and it is stored in plasma membrane associated vesicular structures. Therefore, it has been proposed that DAO might be of primary importance for inactivation and scavenging of extracellular histamine originating from ingested histamine rich food (Schwelberger et al., 1998; Schwelberger and Bodner, 1998; Klocker et al., 2005). On the contrary, HNMT a cytosolic protein with high expression in liver, kidney, lymph nodes, eye and lung can convert histamine only in the intracellular space of cells (De Santi et al., 1998; Klocker et al., 2005). High doses of alimentary histamine and malfunction or reduced activity of DAO can result in high histamine blood levels, which consequently overload the internal hepatic inactivation system of HNMT (Jarisch, 2004). A number of drugs containing DAO-inhibitors and alcohol is known to reduce DAO activity (Sattler et al., 1988; Sessa et al., 1984). Individual differences in enzyme activities besides varying histamine concentrations in food may account for different tolerance levels (Jarisch, 2004).

#### 3.1.2. Physiological role in humans

Histamine acts both as local hormone and neurotransmitter. Mast cells, blood cells (e.g. basophilic granulocytes and thrombocytes), and neurons in the brain have been found to contain histamine (Forth et al., 2001). By interacting with specific receptors (H1, H2, H3, H4) on target cells, histamine

modulates a variety of functions. Its physiological role includes gastric acid secretion, cell growth and differentiation, circadian rhythm, attention and cognition. In addition, histamine is involved in the onset of allergic reactions by binding onto specific receptors which effect the contraction of smooth muscle cells (intestines, lung, uterus), dilatation of blood vessels and, thus, an efflux of blood plasma into surrounding tissues like mucous membranes and subcutis (Jarisch, 2004; Jørgensen et al., 2007; Rangachari, 1992).

### 3.1.3. Toxic effects in humans

In healthy persons, dietary histamine is rapidly detoxified by amine oxidases but they may develop severe symptoms of **histamine intoxication** as a result of its high amounts ingested with food such as scombroid fish or matured cheese (Taylor, 1986; Lehane and Olley, 2000). Impairment of DAO activity either due to genetic predisposition, gastrointestinal diseases, or due to medication with DAO inhibitors results in histamine excess and leads to **histamine intolerance** causing numerous symptoms mimicking an allergic reaction even after the ingestion of small amounts of histamine tolerated by healthy individuals (Maintz and Novak, 2007). Intoxication is characterized by an incubation period ranging from a few minutes to hours, with symptoms that are usually noticeable for a few hours only. The symptoms of histamine poisoning relate to effects on blood vessels and smooth muscles, and include headache, nasal secretion, bronchospasm, tachycardia, extrasystoles, hypotension, edema (eyelids), urticaria, pruritus, flushing and asthma (Jarisch, 2004; Maintz and Novak, 2007).

Dietary histamine has also been implicated in the pathogenesis of **migraine** in susceptible individuals suffering from DAO deficiencies (Maintz and Novak, 2007). Histamine-rich food has been reported by migraine patients to trigger headache which are alleviated by a histamine-free diet (Wantke et al., 1993).

#### 3.1.3.1. Dose-response relationships

Only a limited number of studies have been reported on the dose-response relationships of alimentary histamine, principal data originating either from volunteer studies or from clinical cases, albeit that the findings are not always conclusive (Rauscher-Gabernig et al., 2009).

Healthy volunteers exhibited no symptoms after consumption of 25 – 50 mg histamine with solid food like fish or non-alcoholic drinks (Motil and Scrimshaw, 1979; Van Gelderen et al., 1992; Lüthy and Schlatter, 1983). In some volunteers histamine levels ranging from 75 to 300 mg in fish or non-alcoholic beverages could provoke mostly headache and flushing (Motil and Scrimshaw, 1979; Van Gelderen et al., 1992; Clifford et al., 1989; Wöhrle et al., 2004). In trials with histamine containing alcoholic beverages like wine no significant effects were observed in healthy volunteers, whereas 12 out of 40 patients with histamine intolerance demonstrated clear symptoms like dizziness, headache, nausea and itching (Lüthy and Schlatter, 1983; Menne et al., 2001; Table 1).

Instillation of 120 mg histamine into the duodenum was well tolerated in healthy volunteers; in patients with chronic urticaria symptoms such as urticaria, headache, tachycardia, hypotension, nausea and diarrhoea were observed (Kanny et al., 1993; 1996; Table 1).

**Table 1:** Dose-response relationship of histamine in humans after oral administration

Administration	Histamine *amount ingested	Symptoms	Number of subjects showing symptoms / total number of subjects	Reference
<i>Solid foods</i>				
tuna	25 mg	no symptoms	0/8 healthy volunteers	Motil and Scrimshaw, 1979
herring paste	45 mg	no effect level	0/8 healthy volunteers	Van Gelderen et al., 1992
tuna	50 mg	no symptoms	0/8 healthy volunteers	Motil and Scrimshaw, 1979
herring paste	90 mg	warm face, flushing, headache	2/8 healthy volunteers	Van Gelderen et al., 1992
tuna	100 mg	mild headache, flushing	1/8 healthy volunteers	Motil and Scrimshaw, 1979
tuna	150 mg	mild headache, flushing	2/8 healthy volunteers	Motil and Scrimshaw, 1979
tuna	180 mg	mild to severe headache, flush	4/8 healthy volunteers	Motil and Scrimshaw, 1979
mackerel	300 mg	headache, flushing, oral tingling (no significant effects)	n.i. <sup>a</sup> /7 healthy volunteers	Clifford et al., 1989
<i>Non-alcoholic drinks</i>				
apple juice	25 mg	no statistically significant effects	n.i. <sup>a</sup> /25 healthy volunteers and 2 migraine patients	Lüthy and Schlatter, 1983
grapefruit juice	25 mg	no significant effect	0/4 healthy volunteers	Motil and Scrimshaw, 1979
grapefruit juice	50 mg	no significant effect	0/4 healthy volunteers	Motil and Scrimshaw, 1979
peppermint tea	75 mg	diarrhoea, headache, sneezing, flatulence	5/10 healthy females	Wöhrle et al., 2004
grapefruit juice	100 mg	mild headache, flushing	2/4 healthy volunteers	Motil and Scrimshaw, 1979
grapefruit juice	150 mg	mild headache, flushing	2/4 healthy volunteers	Motil and Scrimshaw, 1979
grapefruit juice	180 mg	severe headache, flushing	1/4 healthy volunteers	Motil and Scrimshaw, 1979
<i>Alcoholic drinks</i>				
wine	0.12 - 4.2 mg	no statistically significant effects	n.i. <sup>a</sup> /20 healthy volunteers	Lüthy and Schlatter, 1983
wine	100 mg	no effects	0/2 healthy volunteers	Lüthy and Schlatter, 1983
sparkling wine	4 mg	dizziness, headache, nausea, itching	12/40 patients with histamine intolerance	Menne et al., 2001
<i>Digestive histamine challenge</i>				
Instillation into the duodenum	120 mg	No symptoms	0/8 healthy volunteers	Kanny et al., 1993
Instillation into the duodenum	120 mg	urticaria, headache, accelerated heart rate, drop in blood pressure, nausea, diarrhoea	26/32 patients with chronic urticaria	Kanny et al., 1993; 1996

<sup>a</sup> n.i. = not indicated

### 3.1.3.2. Outbreak data

#### *Fish*

Fish have been incriminated in the majority of incidents of histamine poisoning, whereas tuna, skipjack, and mackerel seem to be the most commonly implicated food types (Taylor, 1985). Histamine is the main toxin involved in scombroid fish poisoning, also called histamine fish poisoning. It is generally associated with high levels of histamine ( $\geq 500$  mg/kg) in fish. Although there is compelling evidence to implicate histamine as the causative agent in histamine fish poisoning, there is not a straightforward dose-response relationship, as spoiled fish containing histamine tends to be more toxic than the equivalent amount of pure histamine dosed orally (Lehane and Olley, 2000). The main evidences supporting the involvement of histamine in histamine fish poisoning are the identical symptoms of histamine poisoning to those of intravenous histamine administration and the efficacy of antihistamines in histamine fish poisoning therapy (Koutsoumanis et al., 2010). Histamine poisoning was also reported at histamine concentrations lower than 500 mg/kg, e. g. in one outbreak tuna burgers with histamine levels of 213 mg/kg were implicated (Becker et al., 2001) and another case of scombroid poisoning was linked to canned anchovies containing 365 mg/kg histamine (NSW Food Authority, 2010). In the European Rapid Alert system for Food and Feed (RASFF) database adverse effects to consumers were recorded at various histamine concentrations in fish samples (reported concentration ranges in different sub-samples of the same sample e.g. 50 – 500 mg/kg, 102 – 180 mg/kg, 27 – 152 mg/kg, of 147 mg/kg, below 5 to 208 and below 10 to 1000 mg/kg; see section 4.1.1.).

Histamine analysis was carried out on 240 fish samples from incidents of histamine fish poisoning in the UK between 1976 and 1986. The symptoms most consistently reported were rash, diarrhoea, flushing, and headache. Of fish samples with more than 200 mg/kg histamine, 94% were from incidents in which histamine poisoning symptoms were characteristic. Based on the interpretation of quantitative histamine analysis of these fish samples the following recommendations were made: fish containing less than 50 mg/kg histamine is rated as normal and safe for consumption, between 50 and 200 mg/kg histamine the fish is regarded as mishandled and possibly toxic, concentrations between 200 and 1000 mg/kg histamine are classified as unsatisfactory and probably toxic, and those above 1000 mg/kg histamine are rated as toxic and unsafe for consumption (Bartholomew et al., 1987; Table 2).

More recently, in a Danish survey of histamine fish poisoning outbreaks and cases, most of them caused by seafood with histamine concentrations of more than 500 mg/kg. Only in 10% of these cases was seafood with less than 500 mg/kg involved. But for 80% of these cases no plate-sample of the fish actually consumed was available, and therefore, only a sample from the same batch of fish was analysed. This is important to point out because the concentration of histamine can vary considerably from fish to fish within a batch and even between different portions of a single fish. Hence, it cannot be excluded that in some situations where seafood with very low concentrations of histamine have been reported to cause histamine fish poisoning, the seafood-sample actually consumed contained a much higher concentration of histamine (Dalgaard et al., 2008).

**Table 2:** Concentrations in fish samples related to histamine fish poisoning

Histamine concentration	Fish quality	Health effects	Reference
<50 mg/kg	normal	safe for consumption	Bartholomew et al., 1987
50-200 mg/kg	mishandled	possibly toxic	Bartholomew et al., 1987
200-1000 mg/kg	unsatisfactory	probably toxic	Bartholomew et al., 1987
≥500 mg/kg	not reported	toxic	Dalgaard et al., 2008
>1000 mg/kg	unsafe	toxic	Bartholomew et al., 1987

### *Cheese*

After fish, cheese is the next most commonly implicated food item associated with histamine poisoning (Stratton et al., 1991; Silla-Santos, 1996). Reported outbreaks include cheeses made from raw as well as from pasteurized milk (Lund et al., 2000). Cheeses that are associated with outbreaks of histamine poisoning were Gouda, Swiss cheese, Cheddar, Gruyere and Cheshire (Doeglas et al., 1967; Taylor et al., 1982; Shalaby, 1996). Several cases of histamine poisoning have been described in patients on isoniazid therapy (Kahana and Todd, 1981; Uragoda and Lodha, 1979). A histamine outbreak related to grated cheese in Spain was reported via the RASFF in 2006 (see Chapter 4.1.1 RASFF data on histamine). The histamine concentrations in cheeses that were implicated in outbreaks ranged between 850 and 1870 mg/kg.

### *Other foods*

Foods other than fish and cheese can contain high concentrations of histamine and its potentiators, such as sauerkraut. But these are rarely reported to be implicated in histamine poisoning. Chicken, shellfish, and ham were implicated in one outbreak each, and for sauerkraut, one incident is reported. But in each of these cases, evidence linking these foods with histamine poisoning was incomplete. For example, the possible incident of histamine poisoning associated with sauerkraut may be questionable, since the only symptom noted was headache with an absence of other clinical symptoms of histamine poisoning, such as urticaria (Taylor, 1985; Mayer and Pause, 1972). No cases of histamine poisoning have implicated sausages as the causative food, even though the products may occasionally contain histamine at levels as high as those observed in some fish related outbreaks (Stratton et al., 1991; see section 4.1.3.1).



Wine is a common cause for food adverse reactions and may elicit a range of allergy-like symptoms including flushing, itching, headache, rhinitis, meteorism, diarrhoea, as well as urticaria and asthma which will mimic hypersensitivity to sulphites. Histamine and other biogenic amines are considered the most important reason for wine intolerance (Konakovsky et al., 2011). In susceptible individuals, histamine intolerance was triggered by the intake of 4 mg histamine due to consumption of 0.2 l of sparkling wine containing 20 mg/l (Menne et al., 2001; see section 4.1.3.1).

Traditionally fermented soy bean products, like Miso and soy sauce, can contain high concentrations of histamine and other biogenic amines. Although neither miso nor soy sauce have been incriminated in an incident of histamine poisoning, many cases in Japan have involved fish that had been seasoned with soy sauce or eaten with miso. Even though the incriminated fish were the source of the majority of histamine, the amines present in the soy sauce or miso may have also contributed to the toxicity of the fish (Stratton et al., 1991).

#### 3.1.3.3. Indication for a threshold level of adverse health effects

The critical endpoint in acute histamine intoxication is an allergy like reaction comprising symptoms like headache, flushing, itching and urticaria. Results from the limited number of studies suggested a potential no-observed-adverse-effect-level (NOAEL) of 50 mg histamine for the symptoms headache and flushing, but this was based on limited number of individuals: 66 healthy and 74 sensitive. Some healthy individuals did not show symptoms at concentrations up to 6 times higher than the NOAEL. Also in some sensitive individuals no negative effects were observed at concentrations up to 2.4 times higher than the NOAEL. Therefore, it was assumed that the NOAEL may be already a conservative approach and no factor for intraspecies variations was applied. The information on outbreak data was not taken into account, since the actual amount ingested leading to acute effects is often unknown and several other factors such as potentiation of acute effects by other biogenic amines, alcohol or medication could not be excluded. The limited published information available suggested a potential acute reference dose (ARfD) of 50 mg of histamine per healthy person.

#### 3.1.4. Community Summary Report (CSR) data on histamine

In the Community Summary Report including foodborne outbreaks in the European Union in 2008, data were collected on a mandatory basis for the eight zoonotic agents and on a voluntary basis data concerning compliance with microbiological criteria were also reported for histamine (EFSA, 2010a). In this report data concerning 'other causative agents' include histamine, marine biotoxins and mushroom toxins as well as unspecified toxins are presented.

Seventeen MSs reported a total of 167 food-borne outbreaks due to 'other causative agents' (histamine, marine biotoxins and mushroom toxins) which constituted 3.1% of the total number of reported outbreaks. Two non-MSs reported two outbreaks. In 2008, outbreaks occurring in France accounted for 37.7% of 'other causative agents' outbreaks. Of the reported outbreaks caused by 'other causative agents' 68 (40.7%) were verified. However, detailed information on outbreaks was only available for 45 outbreaks because some MSs reported aggregated data (23 outbreaks).

The verified outbreaks were primarily histamine outbreaks in France and Spain. The type of evidence verifying the outbreaks was detection of the agent in implicated foodstuffs in 32 outbreaks and analytical epidemiological evidence was presented in 45 of the outbreaks. Several MSs reported more than one type of evidence. Histamine was the responsible agent for 37 outbreaks in five Member States involving 166 cases of which 12 were hospitalised (Table 3).

**Table 3:** Verified food-borne outbreaks caused by histamine in 2008 (source: EFSA, 2010a)

Country	Number of outbreaks	Human cases number	Human cases hospitalised	Number of deaths
Belgium	1	2	2	0
France	18	82	6	0
Germany	2	6	0	0
Hungary	1	3	3	0
Spain	15	73	1	0
<i>EU Total</i>	<i>37</i>	<i>166</i>	<i>12</i>	<i>0</i>
Switzerland	1	11	3	0

Information on the implicated foodstuff was provided in 38 of the verified outbreaks. Seventeen outbreaks were caused by histamine in fish and fish product. For histamine outbreaks, the contributory factors were unprocessed contaminated ingredients (11 outbreaks), storage time (two outbreaks), and inadequate chilling (three outbreaks). Histamine concentrations involved in the outbreaks were not reported.

### 3.2. Tyramine, phenylethylamine and tryptamine

#### 3.2.1. Synthesis and metabolism in humans

The so-called ‘trace amines’ are synthesised in humans from their corresponding amino acids (tyrosine, phenylalanine and tryptophan, respectively) by decarboxylation. Their catabolism is mainly mediated by monoamine oxidase (MAO). Two MAO isozymes exist, A and B, with different locations and substrate specificity. MAO-A predominates in the stomach, intestine and placenta and has polar aromatic amines (as noradrenalin and octopamine) as preferred substrates. MAO-B predominates in the brain and selectively deaminates non-polar aromatic amines (as phenylethylamine and dopamine). Tyramine is a substrate for either form of MAO, MAO-A is responsible for intestinal metabolism of tyramine, thereby preventing its systemic absorption (Azzaro et al. 2006; Broadley, 2010). Tyramine and phenylethylamine are also subjected to N-methylation by N-methyltransferases, generating the sympathetic neurotransmitter, noradrenaline. Tyramine can be further converted into octopamine (Broadley, 2010).

#### 3.2.2. Physiological role in humans

All biogenic amines are considered a trace amine in humans related to neuromodulating functions, being structurally and functionally associated to catecholamines (Broadley, 2010). They are believed to play a role in human disorders such as schizophrenia, depression, attention deficit disorder and Parkinson’s Disease (Branchek and Blackburn, 2003). The mechanisms of action may depend on their localisation. In the brain, trace amines act as indirect sympathomimetics through the release of noradrenalin, which cause vasoconstriction and transient hypertension. It has been hypothesised that dietary tyramine and trace amines cause vasodilatation of the mesenteric vascular bed, increasing blood flow at the gastrointestinal level and thus facilitating their absorption (Broadley, 2010). Direct effects associated with specific receptors have also been reported at the cardiovascular level, causing an increase in heart rate (Frascarelli et al., 2008).



### 3.2.3. Toxic effects in humans

The vasoconstriction effect of tyramine, phenylethylamine and tryptamine cause hypertension, but other symptoms such as headache, perspiration, vomiting, pupil dilatation, etc. have been described. The clinical signs appear between 30 minutes to a few hours following consumption of biogenic amines and usually disappear within few hours and recovery is usually complete within 24 hours. **Hypertensive crises**, but also in a minority of **migraine cases**, caused by the consumption of food potentially rich in tyramine have been recorded in scientific publications and databases about food and drug interactions particularly with monoamino oxidase inhibitor (MAOI) drugs (e.g. Stockley's Drug Interactions, 2011).

#### 3.2.3.1. Dose-response relationship

The clinical studies dealing with the interaction between dietary tyramine and MAOI drugs allow the assessment of the specific toxicity for the **vasopressor** effect of tyramine in terms of the amount of tyramine needed to provoke a clinically significant increase of systolic blood pressure of at least 30 mmHg (PD30). Studies on phenylethylamine and tryptamine are limited. For the present document, outputs from trials in which tyramine was administered orally accompanying a meal have been taken into consideration, since they are those most closely reproducing the actual situation of food-borne tyramine intake in comparison with pure tyramine in fasting conditions or intravenous injection. Indeed, it has been reported that increasing mainly lipid but also protein content of the meal significantly reduce the tyramine blood pressor response (Audebert et al., 1992). Bioavailability of tyramine when administered with food or as a dietary constituent seems to drastically be reduced and systematic concentrations are reduced by approximately 2- to 3-fold (Patat et al., 1995; Van den Berg et al., 2003; Azzaro et al., 2006).

Information available in the literature is summarised in Table 4. The results from literature showed that from 600 mg up to 2,000 mg of tyramine administered in a meal would be needed to cause a minimal systolic blood pressure increase (of at least 30 mmHg). Therefore the intestinal barriers to tyramine in non-medicated healthy volunteers (placebo groups) seem to be effective to avoid tyramine intoxication from food (Korn et al., 1988a; Berlin et al., 1989; Zimmer et al., 1990; Patat et al., 1995). A dose-response curve was generated by Patat et al. (1995), in which 1100 mg of tyramine correspond to the effective dose (ED<sub>50</sub>) as the tyramine dose at which 50% of the individuals responded. According to Bieck and Antonin (1988) intraindividual coefficient of variation was around 10% in repeated tests and there was no correlation with sex, age or weight.

The situation is different in those individuals medicated with MAOI drugs. The impaired function of MAO enzyme does not allow a complete metabolism of tyramine, leading to elevated plasma tyramine levels after ingestion of tyramine-rich food. In these cases, much less dietary tyramine is needed to produce similar effects (dose-response curve considerably shifts to the left lowering the ED<sub>50</sub> value). The intensity of the MAOI enhancing action depends on the nature of the drugs. A high potentiating factor, from 20 to 56-fold increase in tyramine sensitivity, has been described for the first generation classical MAOIs (irreversible and non-selective, such as tranlylcypromine) (Korn et al., 1988b; Bieck and Antonin, 1989). However, the presently most commonly used new generation drugs represent a progress in safety, since their action is reversible and/or selective for the MAO-A or MAO-B isoenzyme. In these cases, the increase in tyramine sensitivity has been reported to range from 2-5-fold (selegiline), 5-7-fold (moclobemide) to 13-fold (phenelzine) depending on the MAOI dosage (Prasad et al., 1988; Bieck and Antonin, 1988; Bieck and Antonin, 1989; Berlin et al., 1989; Zimmer et al., 1990). According to the literature, 6 mg of tyramine could provoke mild crisis and 10 - 25 mg severe headache with intracranial haemorrhage in patients treated with classical MAOI (McCabe, 1986), whereas from 50 up to 150 mg of tyramine would be well tolerated by patients under new generation MAOI treatment, so called RIMA (reversible inhibitors of MAO-A) (Korn et al., 1988b; Dingemanse et al., 1998; Patat et al., 1995).

**Table 4:** Dose-response relationship of tyramine in humans after oral administration with food (experiments performed in fasting conditions were not taken into account).

Tested sample	Tyramine dose	Hypertensive effects*	Reference
<b>Healthy subjects (placebo groups)</b>			
n=8	50-150 mg	No effect	Dingemanse et al. (1998)
n=5	600 mg	No effect	Barrett et al. (1997)
n=8	100-800 mg	No effect for n=4/8	Provost et al. (1992)
	800 mg	PD30 for n=4/8	
n=16	1200 mg (1000 – 1600 mg)	Mean PD30 (range PD30)	Berlin et al. (1989)
n=30	1100 mg 1220 mg (600-1800 mg)	ED <sub>50</sub> mean PD30 (range PD30)	Patat et al. (1995)
n=16	1450 mg (800 – 2000 mg)	mean PD30 (range PD30)	Berlin et al. (1989)
<b>Subjects under treatment with classical MAOI drug</b>			
<b>Tranylcypromine</b> (20 mg/day); n=16	35 mg (20 – 50 mg)	mean PD30 (range PD30)	Berlin et al. (1989)
<b>Subjects under treatment with RIMA (reversible MAO-A inhibitors)</b>			
<b>Brofaromine</b> (150 mg/day); n=10	25 -75 mg	No effect	Bieck & Antonin (1988)
<b>Moclobemide</b> (450 mg bid) n=8	50 mg	No effect	Dingemanse et al. (1998)
<b>Moclobemide</b> (3x200 mg/day); n=6	200 mg	PD30 for n=2/6 Tolerated by n=4/6	Korn et al. (1988a)
<b>Moclobemide</b> (3x100 mg/day) n=6	150 mg 200 mg 250 mg 300 mg	PD30 for n=1/6 PD30 for n=1/5 PD30 for n=1/3 Tolerated by n=1/1	Korn et al. (1988b)
<b>Moclobemide</b> (450 mg bid); n=8	100 mg 150 mg	PD30 for n=3/8 PD30 for n=1/8	Dingemanse et al. (1998)
<b>Moclobemide</b> 600 mg bid; n=8	50 mg 100 mg 150 mg	PD30 for n=1/8 PD30 for n=4/8 Tolerated by n=1/8	Dingemanse et al. (1998)
<b>Moclobemide</b> (600 mg/day); n=8	250 mg (150-400 mg)	mean PD30 (range PD30)	Audebert et al. (1992)
<b>Moclobemide</b> (200 mg t.i.d); n=16	306 mg (150 – 500 mg)	mean PD30 (range PD30)	Berlin et al. (1989)
<b>Befloxadone</b> (20 mg twice/day) n=10	120 mg 150 mg (100 – 250 mg)	ED <sub>50</sub> mean PD30 (range PD30)	Patat et al. (1995)
<b>Befloxadone</b> (10 mg twice/day) n=10	200 mg 250 mg (100 – 300 mg)	ED <sub>50</sub> mean PD30 (range PD30)	Patat et al. (1995)
<b>Befloxadone</b> (20 mg once daily); n=10	250 mg 290 mg (150 – 500 mg)	ED <sub>50</sub> mean PD30 (range PD30)	Patat et al. (1995)
<b>Toloxatone</b> (n=8)	≤ 200 mg	No effect	Provost et al. (1992)
<b>Toloxatone</b> (400 mg 3-times/day) (n=8)	400 mg 800 mg	Increase in blood pressure PD30 for n=2/8 PD30 for n=5/6	Provost et al. (1992)
<b>Toloxatone</b> (200 mg 3-times/day); (n=8)	800 mg	Increase in blood pressure PD30 for n=4/8	Provost et al. (1992)
<b>Subjects under treatment with irreversible inhibitors of MAO-B</b>			
<b>Selegiline</b> , (transdermal system 12mg/day); n=8	172 mg (75-300 mg)	mean PD30 (range PD30)	Azzaro et al. (2006)

\*No effect: no increase (or less than 30 mmHg increase) in systolic blood pressure

ED<sub>50</sub>: median effective dose (dose affecting 50% of tested population sample)

PD30: pressor doses of tyramine to raise systemic blood pressure by 30 mmHg (minimal pressor response)

Tyramine has also been incriminated as a causative agent of certain food-induced **migraines**, together with phenylethylamine. However, migraine is a multifactorial problem, not only affected by one component of the diet but also by other environmental, physiological and psychological factors. However, only limited data from previous research is available on the migraine triggering threshold dose of biogenic amines. Hannington (1967) pioneered by conducting several challenge studies. He concluded that the ingestion of 125 mg of tyramine was responsible for an overall headache rate of 80%, compared with 8% in case of the placebo (lactose) ingestion. According to Jansen et al. (2003) the experimental design of their studies was not sufficiently accurate and the results can not be considered conclusive. Similar effects occur with the amount of phenylethylamine presumably causing migraine. In healthy volunteers, 5 mg may provoke headache, dizziness and discomfort according to Lüthy and Schlatter (1983). In migraine sufferers, the activity of the MAO-B isoenzyme (main responsible for phenylethylamine oxidation) seems significantly reduced and 3 mg were reported to potentially trigger an attack (Sandler et al., 1974). No dose-response curve has been estimated.

#### 3.2.3.2. Cases of food and drug interactions

Information about adverse health effects caused by dietary tyramine is scarce, although some sporadic cases of food and MAOI drug interaction can be found in the scientific medical literature. Several types of food have been incriminated in these types of interactions, cheese being the most frequently implicated product, although yeast extract, pickled herrings, avocados, beef livers, chicken livers, caviar, soused herrings, tinned fish, tinned milk, peanuts, soy sauce, miso, powdered protein diet supplement, packet soup, sour cream, prickly spinach and beer have also been recorded (Stockley's Drug Interactions, 2011). Unfortunately, the occurrence of tyramine is not always demonstrated in the investigated products and in many other cases the amount of tyramine is not reported.

### 3.3. Putrescine and Cadaverine

#### 3.3.1. Synthesis and metabolism in humans

Putrescine and cadaverine are diamines that can be formed either as natural polyamines during *de novo* polyamine biosynthesis or as biogenic amines by decarboxylation (Bardocz, 1995). Putrescine formation in animals and microorganisms requires the free amino acid ornithine and the enzyme ornithine decarboxylase. Alternatively putrescine can be produced from arginine via agmatine and carbamylputrescine (Lucas et al. 2007).

The amino acid lysine is decarboxylated by lysine decarboxylase to form cadaverine. Different bacteria species exhibit decarboxylases with high activity. During degradation putrescine and cadaverine are oxidised by diamine oxidases to form aldehydes (Askar and Treptow, 1986).

#### 3.3.2. Physiological role in humans

In humans, putrescine acts as a precursor for the physiological polyamines (i.e. spermidine and spermine) and all are involved in the regulation of cell growth, cell division and tumour promotion (Halasz and Barath, 2002). Putrescine is an important constituent of all mammalian cells and is involved essentially in a variety of regulatory steps during normal, adaptive, and malignant cell proliferation. Because of high proliferation rates, intestinal and colonic mucosa has a special demand for putrescine (Löser et al., 1999). The growth of murine colon tumour cells is also stimulated by newly incorporated putrescine (Farriol et al., 2001).

The role of cadaverine is less known but it could also contribute with the above mentioned diamines and polyamines. It has been reported that in some systems, including bacteria, cadaverine can replace putrescine (Hölttä and Pohjanpelto, 1983).

Experiments with rats showed that putrescine is readily taken up from the gut lumen, probably by passive diffusion. More than 80% of the putrescine was converted to other polyamines and non-polyamine metabolites, mostly to amino acids (Bardocz et al., 1995).

### 3.3.3. Toxic effects in humans

The pharmacological activity of putrescine and cadaverine seems to be less potent than those for histamine and tyramine. Adverse effects described are hypotension, bradycardia, lockjaw and paresis of the extremities (Shalaby, 1996; Halász and Barath, 2002). Perhaps, the most relevant issue of putrescine and cadaverine in relation to food is the potentiation of the toxicity of other amines, especially histamine (Hui and Taylor, 1985; Chu and Bjeldanes, 1981) (see 3.3.5.). Finally, these diamines can react with nitrite to form carcinogenic nitrosamines (Nebelin et al., 1980).

#### 3.3.3.1. Dose-response relationship

In contrast to histamine, tyramine and phenylethylamine no human dose-response data are available for alimentary putrescine and cadaverine and only a limited number of animal studies have been published.

In a subacute oral toxicity study with Wistar rats, the no-observed-adverse-effect level (NOAEL) was found to be 180 mg/kg body weight/day for cadaverine and putrescine. Mean body weights, food intake and food efficiency were slightly decreased with the highest doses for cadaverine and putrescine. The relative brain weights were significantly increased with both putrescine and cadaverine. A significant increase in the relative weights of testes was observed with cadaverine only. A dose-related decrease in blood pressure was caused by cadaverine and putrescine after intravenous administration only (Til et al., 1997).

#### 3.3.3.2. Potentiation of the toxicity of other biogenic amines

The amine oxidizing enzymes monoamine oxidase and diamine oxidase are able to metabolise more than one biogenic amine, therefore, the degradation of alimentary biogenic amines is retarded depending on their number and concentration (Beutling, 1996). Hence, the toxicity of biogenic amines will depend on quantitative and qualitative factors associated with the food itself. Additionally, factors like individual susceptibility and health status of consumers are relevant.

Based on *in vitro* and *in vivo* studies, two possible theories were developed about the potentiation of histamine toxicity. According to the theory of competitive inhibition, simultaneous oral intake of other amines, like putrescine and cadaverine, may inhibit the biogenic amine-detoxifying enzymes and consequently block histamine and tyramine metabolism. Though the potentiating mechanism would be equivalent for both histamine and tyramine, most of the published studies refer to histamine. *In vitro* diamines potentiate contractions of the guinea-pig ileum caused by histamine and inhibit its enzymatic destruction by DAO (Mongar, 1957). In rats, metabolism of histamine is inhibited by simultaneous oral administration of cadaverine, putrescine or a mixture of biogenic amines. An increase in the amount of unmetabolized histamine is seen in the 4-hr urine samples. In the presence of the mixture, inhibition is more effective than with single administration (Hui and Taylor, 1985). Besides competing for the same enzymes, the barrier disruption theory suggests that the potentiators such as putrescine and cadaverine may alter the physical barrier function of the small intestine and allow diffusion of greater quantities of histamine into the circulation. *In vitro* studies with pig gastric mucin showed that histamine binding is decreased by cadaverine, putrescine and by a mixture of diamines and polyamines (Chu and Bjeldanes, 1981). This effect was reported to occur only when the ratio between diamines and vasoactive amines is 5:1 or higher (Hui and Taylor, 1985).

In experiments with healthy volunteers no correlation was found between the occurrence of symptoms and the concentration of biogenic amines in food samples containing several biogenic amines in

varying amounts (Lüthy and Schlatter, 1983; Clifford et al., 1991; Van Gelderen et al., 1992). Further, from the scombroid poisoning outbreaks very low levels of diamines have been detected which are unlikely to contribute to histamine potentiation (Emborg et al., 2006; Emborg and Dalgaard, 2006). Thus, no levels of putrescine and cadaverine in food can be estimated causing acute health effects due to the potentiation of histamine toxicity (Joosten, 1988; Hungerford, 2010).

### **3.4. Factors increasing sensitivity to biogenic amines**

In general, increased sensitivity against biogenic amines is due to a weakened enzymatic amine degradation caused by genetic or acquired impairment of MAO, DAO, HNMT function.

It was shown that in patients with food allergy, defined as an immune-mediated hypersensitivity to ingested allergens, average intestinal DAO activity was significantly lower than in healthy individuals (Raithel et al., 1999). Patients suffering from histamine intolerance also exhibit impaired histamine degradation based on reduced diamine oxidase activity or even a lack of the enzyme (Jarisch, 2004). It has been estimated that 1% of the population has histamine intolerance, 80% of those are female middle-aged patients (Jarisch, 2004; Maintz and Novak, 2007).

Certain physiological status can also modify the sensitivity against biogenic amines. In women, a premenstrual decrease in the activity of B-type MAO may cause hypersensitivity to both histamine and tyramine (Bardocz, 1995). Clinical observations indicate that women are more sensitive to histamine on days 12 to 16 of the menstrual cycle (Kalogeromitros et al., 1995). By contrast a physiological increase of DAO production (up to 500-fold) has been reported in pregnant woman, which would explain remissions of food intolerance frequently observed during pregnancy (Jarisch, 2004; Mainz and Novak, 2007).

Individuals with chronic urticaria, atopic eczema, respiratory and coronary problems or those suffering from hypertension or vitamin B6 deficiency are particularly at risk because of their sensitivity to lower doses of biogenic amines (Borysiewicz and Krikler, 1981; Russell and Maretić, 1986; Taylor et al., 1989; Bardocz, 1995; Maintz et al., 2006; Mainz and Novak, 2007).

People with gastro-intestinal problems with altered enterocytes as well as inflammatory and neoplastic diseases (gastritis, irritable bowel syndrome, Crohn's disease, colorectal neoplasmas, stomach and colon ulcers) may be at risk because of the lower activity of oxidases in the intestine compared to healthy individuals (Bardocz, 1995; Raithel et al., 2003; Jarisch, 2004; Mainz and Novak, 2007).

Acquired sensitivity to biogenic amines can be transient and reversible after the elimination of causes. Individuals on medication which acts as a blocker of MAO or DAO activity can be affected; as such drugs prevent the elimination of amines. These MAO and DAO inhibitors include a variety of drugs used for the treatment of stress, depression, Alzheimer's and Parkinson's disease (Sattler et al., 1988; Bardocz, 1995) as well as painkillers, antihypertensive drugs, mucolytics, antibiotics, and agents reducing gut motility, etc. (e.g. acetylcysteine, clavulanic acid, metoclopramide, verapamil, isoniazide, cephalosporines, etc.) (Mainz and Novak, 2007). Indeed, Sattler et al. (1988) estimated that approximately 20% of population would be taking such DAO inhibiting drugs. Typical symptoms of tyramine intoxication can be found in 15% of the population due to genetic enzyme defect or medication (Beutling, 1996).

It has also been reported that tobacco smoke reduce MAO levels by up to 40% and several cigarette smoke compounds can also inhibit MAO enzyme activities (Broadley, 2010).

The capability of detoxifying enzymes (MAO, DAO, HNMT) to degrade ingested amines may be reduced by food components, such as other amines, alcohol and its metabolite acetaldehyde, phenols, etc., acting as potentiators (Hui and Taylor, 1985; Zimatkin and Anichtchik, 1999).



## 4. Exposure assessment related to fermented foods

### 4.1. Occurrence data

#### 4.1.1. RASFF data on histamine

The Rapid Alert System for Food and Feed (RASFF) is a network managed by the European Commission and used by the EU Member States (MSs) for exchange of information on food safety events that do not comply with the existing legislation. As a result of the communication activities, the RASFF Database<sup>8</sup> was established (RASFF, 2010a). It is updated daily in a timely manner. RASFF evolved into a unique collection of food safety events since its creation in 1979. RASFF is a regulatory database reflecting the EU legislation in the field of food and feed safety because an event is notified when there is a breach in the legislation. Changes of the food/feed safety legislation have an impact on the type and the number of the generated notifications. Notifications sent to RASFF depend also on national surveillance programs and the efficiency of national laboratories whereby the approaches are not fully harmonised between MSs. The database includes detailed information on each notification, such as the type and date of notification, the reason for notification (i.e. description of the subject related to the hazard and product under concern), the hazard(s) identified, the nature and traceability of product(s) involved, the country of notification, the country of origin, the laboratory analyses performed with corresponding contamination levels detected, and the size of the consignment.

Biological and chemical hazards such as some biogenic amines are included in the RASFF system. Biogenic amines are represented mainly by histamine in fish. Between January, 2005 and December, 2010 the recorded histamine notifications are 246. The reasons for generating notifications are ‘border controls’ for 119 notifications, ‘official control on the market’ for 80 notifications, ‘food poisoning’ (introduced in RASFF since 2008) for 22, ‘consumer complaint’ for 18, ‘company’s own check’ for five, ‘official control in non-member country’ for two notifications (RASFF, 2010b). People affected by histamine intoxication and reported in RASFF under the search terms ‘consumer complaint’, ‘food poisoning’ and ‘foodborne outbreak’ are 151, distributed as follows: nine consumers in 2005, 31 in 2006, 19 in 2007, 21 in 2008, 38 in 2009 and 33 consumers in 2010 (Table 5). All cases were caused by fish and products thereof. Histamine concentrations were reported for the fish products involved in ‘consumer complaints’ and food poisoning’.

**Table 5:** Consumers affected by histamine intoxication reported in RASFF for the search terms ‘consumer complaint’, ‘food poisoning’ and foodborne outbreaks’ between 2005 to 2010 (source RASFF, 2010a)

Year	Consumer complaint (notified cases)	People affected (number)	Food poisoning (notified cases)	People affected (number)	Foodborne outbreaks (notified cases)	People affected (number)
2005	2	3	0	0	1	6
2006	9	24	0	0	3	7
2007	4	16	0	0	1	3
2008	1	2	5	12	1	4
2009	0	0	12	28	2	10
2010	2	4	5	16	2	13
<i>Total</i>	<i>17</i>	<i>49</i>	<i>22</i>	<i>56</i>	<i>10</i>	<i>43</i>

Actual histamine concentrations in the fish products involved in adverse consumer health effects were reported for ‘consumer complaint’ and ‘food poisoning’ cases and are summarised below (Tables 6, 7).

<sup>8</sup> <https://webgate.ec.europa.eu/rasff-window/portal/>

As can be seen from the data there was sometimes a high variability of concentrations in the same sub sample of up to a factor of ten. Adverse consumer health effects were observed at reported concentrations of around 100 mg/kg histamine in fish.

**Table 6:** Fish products and histamine concentrations and involved for cases notified under ‘consumer complaint’ (source RASFF, 2010a)

Year	Fish product	Histamine concentration (mg/kg)
2005	Fresh tuna fillets	3485
	Tuna in olive oil	8299
2006	Fresh sliced tuna	Not reported
	Tuna chunks in brine	50 – 500
	Fresh chilled tuna	2166; counter analyses 234-848
	Fresh chilled tuna fillets	700
	Frozen vacuum packed tuna chunks	432; 1753
	Tuna steaks	190; >240; 80; 170; 240; >250; >240; >240; >240
	Sliced yellow tuna ( <i>Thunnus albacares</i> )	193.2
	Albacore tuna chunks	1882; 1184
	Tuna loins	4430; 2097
2007	Sardine in soya oil	280 to 1660
	Fresh tuna	Not reported
	Yellow fin tuna loins	102 to 180
	Fresh sliced red tuna	Not reported
2008	Vacuum packed tuna loins ( <i>Thunnus albacares</i> )	496
2010	Fresh tuna ( <i>Thunnus thynnus</i> )	3603
	Chilled tuna loins ( <i>Thunnus albacares</i> )	205



**Table 7:** Fish product and histamine concentrations involved for cases notified under ‘food poisoning’ (source RASFF, 2010)

Year	Fish product	Histamine concentration (mg/kg)
2008	Vacuum packed tuna	5113
	Tuna in sun flower oil	5024
	Chilled vacuum packed tuna fillets ( <i>Thunnus albacares</i> )	1428; 462; 916; 1636; 1443; 1356; 1428; 998; 1993 152; 27; 150; 70; 31; 87; 80; 61; 118
	Smoked tuna in slices	1557
	Vacuum packed tuna loins ( <i>Thunnus albacares</i> )	496
	Canned tuna chunks in brine	500
	Canned tuna fillets in olive oil	1910; 2051; 104; 2066; 2229
2009	Fresh tuna fillets ( <i>Thunnus thynnus</i> )	>1000
	Tuna in sun flower oil	634
	Fresh tuna loins ( <i>Thunnus albacares</i> )	3600
	Fresh tuna loins	1218; 1378
	Yellow fin tuna vacuum packed sashimi loins ( <i>Thunnus albacares</i> )	488
	Raw white sashimi tuna carpaccio	147
	Canned sardine fillets in sun flower oil ( <i>Sardinella aurita</i> )	329; 220; 240; 245
	Escolar fillets ( <i>Lepidocybium flavobrunneum</i> )	<5 to 208 and <50 to 1000
	Fresh tuna fillets ( <i>Thunnus albacares</i> )	Not reported
	Chilled yellow tuna	Not reported
	Fresh yellowtail amberjack ( <i>Seriola lalandi</i> )	1000
2010	Tuna fillets in oil	4398
	Tuna fillets in oil	10000
	Fresh yellow fin tuna loin	1774
	Tuna ( <i>Thunnus alalunga</i> )	3110
	Chilled yellow fin tuna fillets	Not reported

RASFF product categories involved in the histamine notifications are “fish and products thereof” in 228 notifications, “soups, broths and sauces” in 17 notifications and “milk and milk products” in one notification concerning grated cheese. No notifications for histamine or other biogenic amines are reported for meat covered in RASFF by the product categories “meat and meat products (other than poultry)” and “poultry meat and poultry meat products”. In the product category “soups, broths and sauces”, there were 11 notifications concerning fish sauce and six regarding soya sauce. The concentration levels vary from 200 mg/kg to 500 mg/kg in eight notifications, from 500 mg/kg to 1000 mg/kg in eight notifications, from 1000 mg/kg to 2000 mg/kg in one notification. In the product category “milk and milk products” there is only one notification. This report, from 17 November 2006 notified under official control on the market and the hazard histamine, is concerned with grated cheese whereby 850 mg/kg histamine were found and 31 persons were recorded as affected in two related outbreaks. The reasons for generating notifications for non-fish products are official control on the

market for ten notifications and border controls for eight notifications. In 17 out of 18 cases the products concerned were imported into the EU.

#### 4.1.2. Data submitted to EFSA public call for data

The EFSA published in June 2010 on its website a public call for data on the presence of biogenic amines (histamine, tyramine, cadaverine, putrescine, tryptamine, phenylethylamine, spermidine and spermine) in food and beverages. Member States, research institutions, academia and other stakeholders (e.g. meat industry) were specifically invited to submit data.

The data collected are presented in Table 8. Some occurrence data was provided also on amines which were not explicitly listed in the call for data. For example the Teramo University (Italy), provided data also on ethanolamine, ethylamine, isoamylamine, methylamine, serotonin while Budapest University of Technology and Economics provided data on agmatine. A total number of 36,583 analytical results were collected.

Data were submitted using different concentration units such as microgram/gram, milligrams/100 gram, milligram/kilogram, milligram/litre and milligram/millilitre. To summarise the data, the analytical results were all converted to milligram/kilogram (mg/kg). Where the concentrations were expressed in unit of volume, the conversion to unit of mass was performed assuming 1 litre = 1 kilogram. Concentrations have to be expressed in units of mass to be compatible with consumption data that are reported in grams (g).

Data were submitted for the food categories listed in Table 9 including: 'alcoholic beverages', 'coffee', 'composite food', 'sauces', 'fish and fish products', 'fruit', 'juices', 'meat products', 'dairy products', 'vegetables and vegetable products'. To highlight, in particular, the contribution of specific fermented products, these classes were further divided into sub-categories.

'Alcoholic beverages' was divided in 'beer', 'fortified and liqueur wines', 'wine, red', 'wine white sparkling'.

The category 'sauces' was divided in 'fish sauce' and 'other savoury sauces'. The latter includes mainly meat sauce and soya sauces, but these samples have been pooled together given the small number of available samples and the similarity of the occurrence values.

The category 'fish and fish products' was divided into two main sub-categories 'fermented fish' and 'other fish and fish products'. The food items that could be recognised as fermented were classified into the former class. The classification of the reported fish samples was performed based on the 'product text' and 'product treatment' variables of the Standard Sample Description (EFSA, 2010b) reporting format. The category 'other fish and fish products', mainly includes fresh, frozen or canned fish meat not undergoing a fermentation process. Only for histamine, an additional category of food with high values was identified: 'dried anchovies'.

The class 'meat products' has also been split into 'fermented sausages', 'other ripened meat products (including dry-cured ham, dry-cured loin)' and 'other meat products (including cooked ham, mortadella-type of sausages)'. The classes 'fermented sausages' and 'other ripened meat products' were separated since the process of mincing the meat in the 'fermented sausages', allows bacteria to penetrate the surface of the product, which normally is more difficult to occur with a whole muscle. The 'fermented sausages' are therefore expected to show higher values of biogenic amines in comparison to the other classes.

The category 'dairy products' includes mainly cheese. Therefore, a sub-category cheese has been identified. 'Cheese' has been then further divided into four sub-categories: 'fresh cheese', 'hard cheese', 'washed rind cheese' (cheese ripened with bacteria on the rind such as *Brevibacterium linens*),

‘blue cheese’ (cheese with the addition of mould in the curd such as *Penicillium roqueforti*) and acid curd cheese (e.g. Harzer cheese, Mainzer cheese). Although only limited samples are available for acid curd cheese, the high values shown have lead to keep them separate from the other cheese classes. ‘Dairy products’ contains samples of ‘yoghurt’ and ‘other dairy products’ (e.g. cream, sour cream).

**Table 8:** Number of analytical results for biogenic amines by data provider

Country	Organisation providing the data	Biogenic amines								
		Histamine	Tyramine	Putrescine	Cadaverine	Phenylethylamine	Tryptamine	Spermine	Spermidine	Others
Austria	Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH (AGES)	155	29	27	64	30				
	Lebensmittelversuchsanstalt GmbH (LVA)	165								
	University of Natural Resources and Applied Life Sciences Vienna	58	58	58	58	58	58	58	58	
	<i>Total for Austria</i>	378	87	85	122	88	58	58	58	
Cyprus	State General Laboratory		3174							
Germany	LAV Sachsen-Anhalt	100	101	101	101	99		99	99	
Hungary	Central Agricultural Office	97								
	Budapest University of Technology and Economics	439	439	439	439	26	26	439	439	260
	<i>Total for Hungary</i>	536	439	439	439	26	26	439	439	260
Ireland	Food Safety Authority of Ireland (FSAI)	2008	1213	1213	1213	233	233	233	233	
Italy	Stazione sperimentale per l'industria delle conserve alimentari (SSICA)	90	90	90	90		90	90	90	
	University of the Studies of Teramo	28	28	28	28	28		28	28	95
	Istituto Zooprofilattico Sperimentale Umbria e Marche	146								
	<i>Total for Italy</i>	264	118	118	118	28	90	118	118	95
Netherlands	Voedsel en Waren Autoriteit	1922	1922	1922	1922		1922	1922	1922	
Slovakia	Univerzita veterinárskeho lekárstva a farmácie	9	9	9	9					
	Food research Institute	671	246							
	<i>Total for Slovakia</i>	680	255	9	9					
Spain	University of Barcelona	1061	1061	761	761	761	761	761	752	
<i>Total</i>		10123	5196	4648	4685	1235	3090	3630	3621	355

Less than 35 results are available for vegetables, where it is possible to identify some fermented products mainly represented by sauerkraut. The rest of the vegetables comprise data on leafy vegetables and sprouts of different plants.

Less than 7 samples were analysed in the categories ‘cereal products’, ‘chocolate’, ‘coffee’, ‘fruit’ and ‘juices’. The number of samples in these categories was not sufficient to produce a reliable estimation

of biogenic amine occurrence values, therefore they are excluded from further analyses. Also the 'composite food' category was excluded since the class is composed by diverse food sub-classes ranging from pizzas to soups. Again, a sufficient number of samples in these sub-classes is not available to produce reliable statistical estimates. The remaining data provide an adequate overview mainly of fermented products which were the main objective of this opinion. Data on spermidine, spermine and the other amines not explicitly listed in the mandate were also excluded from the final occurrence tables since they are outside the scope of this opinion.

**Table 9:** Number of analytical results for biogenic amines by food class

Food group	Biogenic amines					
	Histamine	Tyramine	Putrescine	Cadaverine	Phenyl-ethylamine	Tryptamine
Alcoholic beverages	786	781	481	487	208	182
Cereal products	7					
Chocolate	1					
Coffee	1	1	1	1		
Composite food	35	12	7	13	4	1
Sauces	99	98	98	99	95	90
Fish and fish products	6454	1351	1349	1359	361	309
Fruit	5	5	5	5		
Juices	3	1	1	1		
Meat products	543	536	536	537	382	429
Dairy products	2154	2388	2147	2160	175	2079
Vegetables and vegetable products	35	23	23	23	10	
Total	10123	5196	4648	4685	1235	3090

Most data submitted were on histamine and fish products since they were generated by control activities required by Commission Regulation (EC) No 2073/2005. Data on cheese and fermented meat instead mainly originated from ad-hoc studies.

Some data senders providing data from control activities on fishery products, in many cases, submitted up to 9 sub-samples, analysed for each data sample as requested by the regulation. After considering that the sampling is frequently done in different parts of a batch, these samples were considered as individual samples.

Data, in general, comprise food samples meant for human consumption taken mainly from retail shops or from border controls and are therefore assumed to be representative of the concentrations of BAs at the point of consumption.

#### 4.1.3. General considerations on occurrence data

The “bounding” approach was applied for non-detected or non-quantified values to reflect the uncertainty originated by these values on the calculated statistics. The lower bound (LB) was obtained by assigning a value of zero to all the samples reported as non-detected or non-quantified. The upper bound (UB) was obtained by assigning the value of the limit of detection (LOD) to values reported as non-detected and the value of the limit of quantification (LOQ) to values reported as non-quantified. Therefore, each reported statistics is presented as interval between the upper and lower bound estimates.

In order to simplify the tables presented in the following paragraphs, the following notation was applied:

- When the lower bound is zero, only the upper bound was reported prefixed by the sign below ‘<’ (e.g. < 0.5).
- When lower bound and upper bound are coincident, only one value was reported (e.g. 0.5).
- When lower bound and upper bound are different both values were reported (e.g. 0.1 – 0.5).

##### 4.1.3.1. Occurrence data for histamine

Table 10 summarises the data received on histamine. Among ‘alcoholic beverages’ the higher levels of histamine were identified for red wine with a mean value between 3.6 and 3.7 mg/kg (or mg/l).

High levels of histamine are present for ‘fish sauce’ with a mean of 196 – 197 mg/kg, while in other type of sauces (e.g. soya and meat) analytical results are mainly non-quantified (96%) with a mean concentration of 0.5 – 10.1 mg/kg. The high spread is due to the percentage of non detected values in this food category.

‘Dried anchovies’ is the subgroup of fish products with highest mean values of histamine (mean 348 mg/kg). This group was listed separately in the Table 10 because of the high occurrence values identified in comparison with the other fish subgroups. The data do not show higher values of histamine in ‘fermented fish’ compared to ‘other fish and fish products’ as expected. This behaviour may be depending on missing information on the fermentation process in some samples or may be linked to issues in the processing or transportation of the raw product. In fact, this second hypothesis is supported by the fact that the six highest results for the class (ranging between 3,100 mg/kg and 8,900 mg/kg) do not include fish that could be potentially fermented but only canned or fresh fish.

For ‘meat products’ the higher values are recorded in ‘fermented sausages’ (mean value of 23.0 – 23.6 mg/kg) followed by ‘other ripened meat products’ (mean value of 5.9 – 6.4 mg/kg). Finally the group ‘other meat products’ shows the lowest concentration inside the broader ‘meat products’ category (mean value of 3.9 - 4.4 mg/kg). ‘Fermented sausages’ shows also high 95-percentiles (P95) of histamine concentration, comparable with the levels shown for some sub-classes of ‘cheese’, although the maximum values reached for ‘cheese’ are not recorded for ‘fermented sausages’.

The class ‘cheese’ shows a high percentage of non-quantified (>80%) results. This causes large gaps between lower bounds (LB) and upper bounds (UB) values. It can be noticed that for the class ‘dairy products’, high values of histamine and in general of biogenic amines, are mainly occurring for cheeses. In fact, both the class ‘yoghurt’ (mean 0.5 mg/kg) and ‘other dairy products’ (mean 0.3 mg/kg) show lower occurrence values.

Finally, the class ‘fermented vegetables’ shows higher mean values for histamine (39.4 – 42.6 mg/kg) in comparison with the class ‘other vegetables’ (2.9 -3.1 mg/kg).

In summary, the food categories showing the highest mean values of histamine are: ‘dried anchovies’ (348 mg/kg), ‘fish sauce’ (196-197 mg/kg), ‘fermented vegetables’ (39.4 - 42.6 mg/kg), ‘cheese’ (20.9 – 62 mg/kg), ‘other fish and fish products’ (26.8 - 31.2 mg/kg) and ‘fermented sausages’ (23.0 – 23.6 mg/kg) .

**Table 10:** Occurrence data on histamine (mg/kg)

Food category	Sub-category	n	ND	Mean	P5	Median	P95	Max
Alcoholic beverages	Beer	188	9.6%	1.4	<0.5	0.7	4.8	21.6
	Fortified and liqueur wines	28	32%	1.1	<0.1	0.7	2.8	2.9
	Wine, red	300	10%	3.6 – 3.7	<0.1	1.4 – 1.5	12.3 – 12.4	34.3
	Wine, white	225	22%	0.8 – 0.9	<0.1	0.3	2.6	55
	Wine, white, sparkling	45	73%	1	<0.1	<0.1	5.2	9.8
<i>Total for alcoholic beverages</i>		786	18%	2 – 2.1	<0.1	0.6 – 0.7	8.8 – 9.2	55
Sauces	Fish sauce	72	14%	196 – 197	<10	180	597	758
	Other savoury sauces	27	96%	0.5 – 10.1	<10	<10	<13.3	16
<i>Total for sauces</i>		99	36%	142 – 146	<10	108	547	758
Fish and fish products	Dried anchovies	54	50%	348	<0.1	10.4 – 10.5	1440	2860
	Fermented fish products	71	45%	7.7 – 11.4	<1.5	1.5 – 4	34.9	163
	Other fish and fish products	6329	73%	26.8 – 31.2	<0.1	<2.5	60.5 – 100	8910
<i>Total for fish and fish products</i>		6454	73%	29.3 – 33.6	<0.1	<2.5	67.7 – 100	8910
Meat products	Fermented sausages	374	43%	23.0 – 23.6	<0.1	0.6 – 1.4	149	400
	Other ripened meat products	94	28%	5.9 – 6.4	<0.1	1	35	150
	Other meat products	75	80%	3.9 – 4.4	<0.1	<1	4.8	212
<i>Total for meat products</i>		543	45%	17.4 – 17.9	<0.1	0.5 – 1	119	400
Dairy products	Cheese	2143	85%	20.9 – 62	<5.5	<50	130	1850
	Fresh cheese	98	93%	3.2 – 38.5	<0.3	<50	20 – 50	119
	Hard cheese	1067	83%	25.2 – 65.1	<3.5	<50	140	1240
	Washed rind cheese	296	93%	8.5 – 54.4	<50	<50	46 – 50	392
	Blue cheese	678	85%	21.8 – 63.8	<45	<50	153	1850
	Acid curd cheese	4	25%	51.3 – 55.3	<9.1	51.6 – 55	102	102
	Yoghurt	7	14%	0.5	<0.1	0.4	1	1
	Other dairy products	4	50%	0.3	<0.1	0.2	0.6	0.6
<i>Total for dairy products</i>		2154	85%	20.8 – 61.6	<4	<50	130	1850
Vegetables and vegetable products	Fermented vegetables	9	44%	39.4 – 42.6	<4	61	92	92
	Other vegetables	26	96%	2.9 – 3.1	<0.1	<0.3	<0.5	75.7
<i>Vegetables and vegetable products</i>		35	83%	12.3 – 13.3	<0.1	<0.5	77	92

The statistics are presented using a bounded approach for the handling of non-detected/non-quantified data, therefore they are displayed as ranges. The upper bound of the range estimates the non-detected/non-quantified values using the reported limit of detection (LOD) or limit of quantification (LOQ) respectively. The lower bound of the range instead assumes the non-detected/non-quantified values as zero. When the lower bound and the upper bound of the range are coincident, only one number is presented. When the lower bound is zero, the range is represented by the upper bound prefixed by ‘<’. The table contains the number of samples (n), the percentage of non detected (ND), the mean, several percentiles to describe the occurrence distribution (P5, P50 or median, P95 and max).

#### 4.1.3.2. Occurrence data for tyramine

Occurrence data on tyramine are presented in Table 11. Among alcoholic beverages, the highest values of tyramine are reached for ‘beer’ and ‘fortified and liqueur wines’ with a mean value of 6 mg/kg (or mg/L). As for histamine, fish sauce also presents high concentrations of tyramine with a mean of 105 –

107 mg/kg. The ‘other savoury sauces’ have low concentrations with a 93% of non-quantified values providing a measured value for the 95-percentile of 18.6 mg/kg. For tyramine, the categories ‘fish and fish products’, ‘meat products’ and ‘vegetables and vegetable products’, show higher occurrence values for fermented products in comparison with non-fermented ones. The broad category ‘cheese’, ‘acid curd cheese’ (335 mg/kg), ‘hard cheese’ (82.9 – 113 mg/kg) and ‘blue cheese’ (63.2 – 104 mg/kg) present higher mean concentrations compared to the other.

In summary, the food categories showing the highest mean values of tyramine are ‘fermented sausages’ (136 mg/kg), ‘fish sauce’ (105 -107 mg/kg), ‘cheese’ (68.5 – 104 mg/kg), ‘fermented fish’ (47.2 -47.9 mg/kg) and ‘fermented vegetables’ (45 – 47.4 mg/kg).

**Table 11:** Occurrence data on tyramine (mg/kg)

Food category	Sub-category	n	ND	Mean	P5	Median	P95	Max
Alcoholic beverages	Beer	188	0%	6.1	1.4	3.2	24.7	46.8
	Fortified and liqueur wines	28	3.6%	6	0.1	1.5	21.3	22.5
	Wine, red	296	12%	2.7 – 2.9	<0.2	1.6 – 1.8	7.8 – 8.5	18.5
	Wine, white	224	17%	1.1 – 1.2	<0.1	0.8	4.3 – 4.5	10
	Wine, white, sparkling	45	56%	4.9	<0.1	<0.1	26.4	47.3
<i>Total for alcoholic beverages</i>		781	13%	3.3 – 3.4	<0.1	1.7	11.5 – 11.6	47.3
Sauces	Fish sauce	71	15%	105 – 107	<10	69.3	421	741
	Other savoury sauces	27	93%	1.5 – 10.5	<10	<10	18.6	21
<i>Total for sauces</i>		98	37%	76.7 – 80.4	<10	55	355	741
Fish and fish products	Fermented fish meat	70	26%	47.2 – 49.1	<4.5	17.4 – 18	251	627
	Other fish and fish products	1281	86%	6.8 – 14.6	<1	<10	33.4	634
<i>Total for fish and fish products</i>		1351	83%	8.9 – 16.4	<1	<10	43.3	634
Meat products	Fermented sausages	369	22%	136	<0.1	99	397	1740
	Other ripened meat products	92	18%	44 – 44.2	<0.3	25.7	149	387
	Other meat products	75	27%	16.1 – 16.2	<0.1	4.9	67	292
<i>Total for meat products</i>		536	22%	104	<0.1	49	361	1740
Dairy products	Cheese	2377	75%	68.5 – 104	<5	<50	440	2130
	Fresh cheese	98	91%	12.8 – 48	<0.3	<50	89	457
	Hard cheese	1303	66%	82.9 – 113	<5	<50	475	1450
	Washed rind cheese	296	92%	31.6 – 76.1	<50	<50	240	1900
	Blue cheese	676	83%	63.2 – 104	<50	<50	453	2130
	Acid curd cheese	4	0%	335	100	380	480	480
	Yoghurt	7	29%	1.9	<0.1	1	5.2	5.2
	Other dairy products	4	25%	0.3	<0.1	0.3	0.4	0.4
<i>Total for dairy products</i>		2388	75%	68.1 – 103	<5	<50	433	2130
Vegetables and vegetable products	Fermented vegetables	9	22%	45 – 47.4	<5	44	91	91
	Other vegetables	14	93%	1.8	<0.1	<0.1	25.4	25.4
<i>Total for vegetables and vegetable products</i>		23	65%	18.7 – 19.7	<0.1	<0.1	91	91

The statistics are presented using a bounded approach for the handling of non-detected/non-quantified data, therefore they are displayed as ranges. The upper bound of the range estimates the non-detected/non-quantified values using the reported limit of detection (LOD) or limit of quantification (LOQ) respectively. The lower bound of the range instead assumes the non-detected/non-quantified values as zero. When the lower bound and the upper bound of the range are coincident, only one number is presented. When the lower bound is zero, the range is represented by the upper bound prefixed by ‘<’. The table contains the number of samples (n), the percentage of non detected (ND), the mean, several percentiles to describe the occurrence distribution (P5, P50 or median, P95 and max).



#### 4.1.3.3. Occurrence data for putrescine

Descriptive statistics of data regarding putrescine are provided in Table 12. For ‘alcoholic beverages’ the highest mean values of putrescine concentrations are present for ‘wine, white, sparkling’ (5.2 mg/kg or mg/l), followed by ‘wine, red’ (4.2 – 4.8 mg/kg of mg/l) and ‘beer’ (3.3 – 3.5 mg/kg or mg/l).

**Table 12:** Occurrence data on putrescine expressed in mg/kg

Food category	Sub-category	n	ND	Mean	P5	Median	P95	Max
Alcoholic beverages	Beer	188	32%	3.3 – 3.5	<0.4	3.3	8.3	17.8
	Fortified and liqueur wines	28	0%	1.4	0.3	0.9	3.6	4.3
	Wine, red	120	5.0%	4.2 – 4.8	0.3 – 1	3.4 – 3.7	9.5 – 11.5	21.6
	Wine, white	100	3.0%	1.4 – 1.5	0.2	1	3.9 – 4.3	5.7 – 10
	Wine, white, sparkling	45	11%	5.2	<0.1	2.4	15	46.4
<i>Alcoholic beverages</i>		<i>481</i>	<i>16%</i>	<i>3.2 – 3.4</i>	<i>&lt;0.4</i>	<i>2.3 – 2.4</i>	<i>8.6 – 9</i>	<i>46.4</i>
Sauces	Fish sauce	71	11%	98.1 – 99.3	<10	82	167	1220
	Other savoury sauces	27	70%	6 – 13.6	<10	<10	24.2	27.4
<i>Total for sauces</i>		<i>98</i>	<i>28%</i>	<i>72.7 – 75.7</i>	<i>&lt;10</i>	<i>53.4</i>	<i>163</i>	<i>1220</i>
Fish and fish products	Fermented fish meat	70	41%	13.4 – 17	<1.6	2.5 – 5	75.1	244
	Other fish and fish products	1279	81%	4.1 – 11.7	<0.2	<10	26.4	337
<i>Total for fish and fish products</i>		<i>1349</i>	<i>79%</i>	<i>4.6 – 12</i>	<i>&lt;0.2</i>	<i>&lt;10</i>	<i>26.4</i>	<i>337</i>
Meat products	Fermented sausages	369	24%	84.2 – 84.6	<0.1	26.9	334	1550
	Other ripened meat products	92	3.30%	32.8	1	12.1	136	406
	Other meat products	75	27%	17.4 – 17.6	<0.1	1.2	123	305
<i>Total for meat products</i>		<i>536</i>	<i>21%</i>	<i>66 – 66.3</i>	<i>&lt;0.1</i>	<i>12.9 – 13.9</i>	<i>284</i>	<i>1550</i>
Dairy products	Cheese	2136	82%	25.4 – 65	<6.2	<50	143	1560
	Fresh cheese	98	85%	5.5 – 41.3	<0.2	<50	4 – 50	348
	Hard cheese	1062	82%	26.6 – 65.5	<3.9	<50	132	1560
	Washed rind cheese	296	82%	32.3 – 72.3	<20	<50	182	816
	Blue cheese	676	83%	20.9 – 62.2	<21.8	<50	149	701
	Acid curd cheese	4	0%	449	50	550	648	648
	Yoghurt	7	0%	0.7	0.1	0.8	1.1	1.1
	Other dairy products	4	0%	0.7	0.4	0.8	0.9	0.9
<i>Total for dairy products</i>		<i>2148</i>	<i>82%</i>	<i>25.3 – 64.7</i>	<i>&lt;4.8</i>	<i>&lt;50</i>	<i>143</i>	<i>1560</i>
Vegetables and vegetable products	Fermented vegetables	9	0%	264	33	249	549	549
	Other vegetables	14	7.1%	37.2	<0.1	12.9	310	310
<i>Total for vegetables and vegetable products</i>		<i>23</i>	<i>4.3%</i>	<i>126</i>	<i>1.8</i>	<i>33</i>	<i>415</i>	<i>549</i>

The statistics are presented using a bounded approach for the handling of non-detected/non-quantified data, therefore they are displayed as ranges. The upper bound of the range estimates the non-detected/non-quantified values using the reported limit of detection (LOD) or limit of quantification (LOQ) respectively. The lower bound of the range instead assumes the non-detected/non-quantified values as zero. When the lower bound and the upper bound of the range are coincident, only one number is presented. When the lower bound is zero, the range is represented by the upper bound prefixed by ‘<’. The table contains the number of samples (n), the percentage of non detected (ND), the mean, several percentiles to describe the occurrence distribution (P5, P50 or median, P95 and max).

For ‘fish sauce’ (98.1 – 99.3 mg/kg), similarly to the other amines reported in previous tables, the concentration of putrescine is higher compared to ‘other savoury sauces’ (6 – 13.6 mg/kg). This difference is also present in fish and meat, where ‘fermented fish meat’ (13.4 – 17 mg/kg) and ‘fermented sausages’ (84.2 – 84.6 mg/kg) have higher values in comparison to the other food items in the respective groups.

Among cheese subclasses, slight differences in putrescine concentration were observed.

In the class vegetables, the difference between fermented vegetables (mean value of 264 mg/kg) and 'other vegetables' (mean value of 37.2 mg/kg) was observed (with the exception of 'acid curd cheese' presenting again very high values (mean 449 mg/kg).

In summary, the food categories showing the highest mean values for putrescine were 'fermented vegetables' (264 mg/kg), 'fish sauce' (98.1 – 99.3 mg/kg), 'fermented sausages' (84.2 – 84.6 mg/kg), 'cheese' (25.4 – 65 mg/kg) and 'fermented fish' (13.4 – 17 mg/kg).

#### 4.1.3.4. Occurrence data for cadaverine

The data on cadaverine are presented in Table 13. For 'alcoholic beverages' the highest values of cadaverine were detected for 'beer' with a mean of 1.3 – 1.5 mg/kg (or mg/l). As the other biogenic amines, high values of cadaverine are measured for 'fish sauce'. The mean value ranges between 180 mg/kg and 182 mg/kg. It should be noticed that for the group 'other savoury sauces' (including mainly meat sauce and soya sauce), there is a very high percentage of non-quantified results.

In the category 'fish and fish products' no large difference between fermented and non-fermented items was observed, in particular when looking at the mean. The food item 'other fish and fish products' presented a higher maximum value of 1690 mg/kg which was obtained from a single sample of mackerel where the processing was not specified.

For meat, fermented sausages the highest concentration of cadaverine (mean 37.4 – 38 mg/kg) were reported, followed by 'other ripened meat products' had higher concentrations of cadaverine (mean 17.2 -17.5 mg/kg) with respect to 'other meat products' (6.7 mg/kg – 6.8 mg/kg).

**Table 13:** Occurrence data on cadaverine expressed in mg/kg

Food category	Sub-category	n	ND	Mean	P5	Median	P95	Max
Alcoholic beverages	Beer	188	46%	1.3 – 1.5	<0.4	0.4	5.3	31.4
	Fortified and liqueur wines	28	57%	0.1	<0.1	<0.1	0.3	0.4
	Wine, red	126	26%	0.2 – 0.5	<0.1	0.1 – 0.2	0.6 – 1.6	5
	Wine, white	100	30%	0.1 – 0.2	<0.1	<0.1	0.3 – 0.4	10
	Wine, white, sparkling	45	91%	<0.1	<0.1	<0.1	0.2	0.5
<i>Total for alcoholic beverages</i>		487	43%	0.6 – 0.8	<0.1	<0.3	2 – 3.9	31.4
Sauces	Fish sauce	71	15%	180 – 182	<10	164	502	1150
	Other savoury sauces	28	96%	2.9 – 13	<10	<10	<20	82.1
<i>Total for sauces</i>		99	38%	130 – 134	<10	103	317	1150
Fish and fish products	Fermented fish meat	70	44%	14 – 17.3	<1.3	1.6 – 7.7	34.5	356
	Other fish and fish products	1289	73%	24 – 30.8	<0.2	<10	130	1690
<i>Total for fish and fish products</i>		1359	72%	23.5 – 30.1	<0.2	<10	126	1690
Meat products	Fermented sausages	370	28%	37.4 – 38	<0.1	5.4 – 6.8	154	1250
	Ripened meat products	92	11%	17.2 – 17.5	<0.7	5 – 5.7	84.1	305
	Other meat products	75	21%	6.7 – 6.8	<0.1	2.4	25	64
<i>Total for meat products</i>		537	24%	29.6 – 30.1	<0.1	5 – 5.4	137	1250
Dairy products	Cheese	2149	77%	72 – 109	<4.5	<50	470	3170
	Fresh cheese	98	85%	10.7 – 45	<0.2	<50	33.8 – 50	389
	Hard cheese	1071	76%	47.8 – 83.5	<2	<50	240	3170
	Washed rind cheese	299	78%	146 – 184	<50	<50	989	2460
	Blue cheese	677	77%	83.1 – 121	<44.1	<50	519	3120
	Acid curd cheese	4	0%	628	86	723	980	980
	Yoghurt	7	29%	3.2	<0.1	0.9	10.3	10.3
	Other dairy products	4	0%	1.9	0.6	2	3	3
<i>Total for dairy products</i>		2160	76%	71.7 – 108	<3.1	<50	464	3170
Vegetables and vegetable products	Fermented vegetables	9	56%	26 – 35.4	<17	<17	94	94
	Other vegetables	14	64%	17	<0.1	<0.1	85	85
<i>Total for vegetables and vegetable products</i>		23	61%	20.5 – 24.2	<0.1	<17	85	94

The statistics are presented using a bounded approach for the handling of non-detected/non-quantified data, therefore they are displayed as ranges. The upper bound of the range estimates the non-detected/non-quantified values using the reported limit of detection (LOD) or limit of quantification (LOQ) respectively. The lower bound of the range instead assumes the non-detected/non-quantified values as zero. When the lower bound and the upper bound of the range are coincident, only one number is presented. When the lower bound is zero, the range is represented by the upper bound prefixed by '<'. The table contains the number of samples (n), the percentage of non detected (ND), the mean, several percentiles to describe the occurrence distribution (P5, P50 or median, P95 and max).

Among cheese subclasses, the class 'washed rind cheese' (mean 146 – 184 mg/kg) presented higher values of cadaverine in comparison with the others (with the exception of 'acid curd cheese' which shows again very high values (628 mg/kg)).

Finally, cadaverine shows closer levels of occurrence between 'fermented vegetables' and 'other vegetables' in comparison with the other biogenic amines.

In summary, the food categories showing the highest mean values of cadaverine were ‘fish sauce’ (180 – 182 mg/kg), ‘cheese’ (72 – 109 mg/kg), ‘fermented sausages’ (37.4 – 38 mg/kg), ‘fermented vegetables’ (26 – 35.4 mg/kg) and ‘fermented fish meat’ (14 – 17.3 mg/kg).

#### 4.1.3.5. Occurrence data for phenylethylamine

Phenylethylamine data are presented in Table 14. In several food groups, phenylethylamine has 100% non-quantified values. Among alcoholic beverages, phenylethylamine had quantified values only for beer (65% non-quantified) with a mean 0.3- 0.5 mg/kg. Fish sauce had a mean phenylethylamine concentration between 17.2 mg/kg and 20.9 mg/kg, while for other savoury sauces the values were entirely non-quantified.

**Table 14:** Occurrence data on phenylethylamine expressed in mg/kg

Food class	Sub-category	n	ND	Mean	P5	Median	P95	Max
Alcoholic beverages	Beer	182	65%	0.3 - 0.5	<0.4	<0.4	0.9	8.4
	Wine, red	24	100%	<2.4	<1.5	<1.5	<5	<5
	Wine, white	2	100%	<1.5	<1.5	<1.5	<1.5	<1.5
<i>Total for alcoholic beverages</i>		208	70%	0.2 - 0.7	<0.4	<0.4	0.8 - 1.5	8.4
Sauces	Fish sauce	68	37%	17.2 - 20.9	<10	12.3 - 12.5	58.6	172
	Other savoury sauces	27	100%	<9.6	<5	<10	<10	<10
<i>Total for sauces</i>		95	55%	12.3 - 17.7	<10	<10	44.6	172
Fish and fish products	Fermented fish meat	68	65%	0.9 - 3.9	<0.2	<5	5.7 - 9.2	9.2 - 18
	Other fish and fish products	293	89%	2.3 - 7.1	<0.2	<10	12.2	180
<i>Total for fish and fish products</i>		361	84%	2 - 6.5	<0.2	<5	6.7 - 10.8	180
Meat products	Fermented sausages	297	48%	6.2 - 7	<0.1	0.3 - 2.1	34.7	182
	Other ripened meat products	34	68%	0.5 - 1.9	<0.4	<1	3.3 - 10	3.7 - 10
	Other meat products	51	92%	0.1 - 1.1	<0.6	<1	1 - 1.1	1.4 - 5.5
<i>Total for meat products</i>		382	55%	4.9 - 5.7	<0.1	<1	28.5	182
Dairy products	Cheese	175	66%	3.4 - 5	<0.2	<2	18.8	61.3
	Fresh cheese	23	100%	<1.4	<0.2	<0.2	<5	<18
	Hard cheese	113	59%	4.2 - 5.5	<0.2	<2	22	61.3
	Washed rind cheese	9	100%	<2.9	<2	<2	<10	<10
	Blue cheese	26	46%	5.1 - 5.6	<0.2	1.3 - 2.3	17.4	39.5
	Acid curd cheese	4	100%	<10.8	<2	<11.5	<18	<18
<i>Total for dairy products</i>		175	66%	3.4 - 5	<0.2	<2	18.8	61.3
Vegetables and vegetable products	Fermented vegetables	9	100%	<5	<5	<5	<5	<5
	Other vegetables	1	100%	<9.3	<9.3	<9.3	<9.3	<9.3
<i>Total for vegetables and vegetable products</i>		10	100%	<5.4	<5	<5	<9.3	<9.3

The statistics are presented using a bounded approach for the handling of non-detected/non-quantified data, therefore they are displayed as ranges. The upper bound of the range estimates the non-detected/non-quantified values using the reported limit of detection (LOD) or limit of quantification (LOQ) respectively. The lower bound of the range instead assumes the non-detected/non-quantified values as zero. When the lower bound and the upper bound of the range are coincident, only one number is presented. When the lower bound is zero, the range is represented by the upper bound prefixed by ‘<’. The table contains the number of samples (n), the percentage of non detected (ND), the mean, several percentiles to describe the occurrence distribution (P5, P50 or median, P95 and max).

In fish, phenylethylamine presented lower values in 'fermented fish' (0.9 – 3.9 mg/kg) in comparison with 'other fish and fish products' (2.3 – 7.1 mg/kg).

The class 'fermented sausages' had a mean concentration of 6.2 - 7 mg/kg in comparison to the 0.5 - 1.9 mg/kg for the other meat products.

In cheese, only one sample was available for the food class 'washed rind cheese'. Fresh cheese and acid curd cheese were entirely non-quantified. Quantified values are present only for hard cheese and blue cheese, with a concentration of around 5 mg/kg.

For phenylethylamine in vegetables, only a few samples were reported, entirely non-quantified.

In summary, the food categories showing the highest mean values of phenylethylamine were 'fish sauce' (17.2 - 20.9 mg/kg), 'fermented sausages' (6.2 – 7 mg/kg), 'cheese' (3.4 - 5mg/kg) and 'other fish and fish products' (2.3 - 7.1 mg/kg).

#### 4.1.3.6. Occurrence data for tryptamine

Table 15 contains descriptive statistics for data received on tryptamine. In the 'alcoholic beverages' group data are available only on beer with a mean ranging between 0.2 mg/kg and 0.7 mg/kg.

**Table 15:** Occurrence data on tryptamine expressed in mg/kg

Food class	Sub-category	n	ND	Mean	P5	Median	P95	Max
Alcoholic beverages	Beer	182	86%	0.2 - 0.7	<0.5	<0.5	1.7	5.4
<i>Total for alcoholic beverages</i>		182	86%	0.2 - 0.7	<0.5	<0.5	1.7	5.4
Sauces	Fish sauce	65	54%	88.1 - 93.5	<10	<10	244	2280
	Other savoury sauces	25	100%	<10	<10	<10	<10	<10
	<i>Total for sauces</i>	90	67%	63.7 - 70.3	<10	<10	224	2280
Fish and fish products	Fermented fish meat	36	61%	1 - 1.2	<0.2	<0.2	6.5	10.1
	Other fish and fish products	273	89%	3.1 - 7.7	<0.2	<10	15.5	362
	<i>Total for fish and fish products</i>	309	86%	2.9 - 7	<0.2	<1.9	14.4	362
Meat products	Fermented sausages	286	52%	8.3 - 8.5	<0.1	<1	42.9	194
	Other ripened meat products	78	97%	0.1 - 0.4	<0.2	<0.2	<1	2.9
	Other meat products	65	89%	0.2 - 0.9	<0.2	<1	1.1	5.1
	<i>Total for meat products</i>	429	66%	5.6 - 5.9	<0.1	<1	29.9	194
Dairy products	Cheese	2079	96%	1 - 46.8	<3.3	<50	<50	312
	Fresh cheese	92	98%	0.8 - 38.8	<0.2	<50	<50	40 - 50
	Hard cheese	1021	94%	1.4 - 45.7	<3.3	<50	1.9 - 50	312
	Washed rind cheese	290	99%	0.5 - 49.2	<50	<50	<50	84.5
	Blue cheese	675	98%	0.5 - 48.6	<50	<50	<50	128
	Acid curd cheese	1	0%	134	134	134	134	134
	<i>Total for dairy products</i>	2079	96%	1 - 46.8	<3.3	<50	<50	312

The statistics are presented using a bounded approach for the handling of non-detected/non-quantified data, therefore they are displayed as ranges. The upper bound of the range estimates the non-detected/non-quantified values using the reported limit of detection (LOD) or limit of quantification (LOQ) respectively. The lower bound of the range instead assumes the non-detected/non-quantified values as zero. When the lower bound and the upper bound of the range are coincident, only one number is presented. When the lower bound is zero, the range is represented by the upper bound prefixed by '<'. The table contains the number of samples (n), the percentage of non detected (ND), the mean, several percentiles to describe the occurrence distribution (P5, P50 or median, P95 and max).

In the 'fish and fish products' class, 'fermented fish meat' (1 – 1.2 mg/kg) does not have higher values in comparison to the 'other fish and fish products' (3.1 – 7.7 mg/kg), while among 'meat products' 'fermented sausages' (8.3 – 8.5 mg/kg) had higher values in comparison with 'other ripened meat products' (0.1 – 0.4 mg/kg) and 'other meat products' (0.2 – 0.9 mg/kg).

For tryptamine, the class 'cheese' shows large numbers of non-quantified values in all the sub-categories: The average concentration of tryptamine for this class is less than 50 mg/kg.

In summary, the food categories showing the highest mean values of tryptamine was 'fish sauce' (88.1 - 93.5 mg/kg), 'cheese' (1 - 46.8 mg/kg), 'fermented sausages' (8.3 - 8.5 mg/kg). The large spread in the values for cheese was due to the high percentage of non-quantified values in this class (96 %).

#### 4.1.3.7. Sum of biogenic amines

The sum of biogenic amines can be calculated only for those samples analysed for all relevant substances (Table 16). It is necessary, then, to restrict the number of compounds otherwise the lower number of samples available for tryptamine and phenylethylamine would limit the number of samples available for the calculation of the sum. Therefore, the sum of biogenic amines is taking into account only samples with values for histamine, tyramine, cadaverine and putrescine. In addition, food categories for which the mean values were significantly different from the one provided in the tables above ('wine red', 'wine white' and 'other fish products') were also not included for the calculation of the sum of biogenic amines. Cumulative values for the categories were also excluded for the same reason.

The sum of biogenic amines is presented in the occurrence section for completeness and it was not used for exposure assessment.



**Table 16:** Sum of biogenic amines in samples with available concentrations of histamine, tyramine, putrescine and cadaverine

Food class	Sub-category	n	Biogenic amines									
			Histamine		Tyramine		Putrescine		Cadaverine		Sum of BAs	
			Mean (mg/kg)	P95 (mg/kg)	Mean (mg/kg)	P95 (mg/kg)	Mean (mg/kg)	P95 (mg/kg)	Mean (mg/kg)	P95 (mg/kg)	Mean (mg/kg)	P95 (mg/kg)
Alcoholic beverages	Beer	188	1.4	4.8	6.1	24.7	3.3 - 3.5	8.3	1.3 - 1.5	5.3	12.1 - 12.4	36.7
	Fortified and liqueur wines	28	1.1	2.8	6	21.3	1.4	3.6	0.1	0.3	8.6	26.4
	Wine, white, sparkling	45	1	5.2	4.9	26.4	5.2	15	<0.1	0.2	11.1	46.1
Condiment	Fish sauce	71	198 - 199	597	105 - 107	421	98.1 - 99	167	180 - 182	502	582 - 588	1500
	Other savoury sauces	27	0.5 - 10.1	<13.3	1.5 - 10	18.6	6 - 13.6	24.2	3 - 12.7	<17	11 - 47	24.2 - 56
Fish and fish products	Fermented Fish products	68	7.7 - 11.4	31.5	45.5 - 47	136	12.2 - 15	75.1	14.4 - 17	34.5	79.8 - 91	552 - 572
Meat products	Fermented sausages	369	23.2 - 23	149	136	397	84.2 - 84	334	37.4 - 38	154	281 - 283	889
	Other ripened meat products	92	6 - 6.2	35	44 - 44.2	149	32.8	136	17.2 - 17	84.1	100 - 101	342
	Other meat products	75	3.9 - 4.4	4.8	16.1 - 16	67	17.4 - 17	123	6.7 - 6.8	25	44 - 45	151
Dairy products	Cheese	2136	20.6 - 61	127	59 - 98	420	25.4 - 65	143	72.2 - 109	472	177 - 334	1050
	Fresh cheese	98	3.2 - 38	20 - 50	12.8 - 48	89	5.5 - 41	4 - 50	10.7 - 45	33.8 - 50	32.1 - 172	323 - 464
	Hard cheese	1062	25 - 65	136	67.1 - 103	475	26.6 - 65	132	47.8 - 83	235	167 - 318	940 - 1030
	Washed rind cheese	676	8.5 - 54	46 - 50	31.6 - 76	240	32.3 - 72	182	147 - 186	989	220 - 388	1420 - 1516
	Blue cheese	296	21.3 - 63	149	63.2 - 10	453	20.9 - 62	149	83.1 - 12	519	188 - 351	1100 - 1184
	Acid curd cheese	4	51.3 - 55	102	335	480	449	648	628	980	1460	2140
	Yoghurt	7	0.5	1	1.9	5.2	0.7	1.1	3.2	10.3	6.3	12
	Other dairy products	4	0.3	0.6	0.3	0.4	0.7	0.9	1.9	3	3.1	4.8
Vegetables and vegetable products	Fermented vegetables	9	39.4 - 42	92	45 - 47.4	91	264	549	26 - 35.4	94	375 - 390	747
	Other vegetables	14	5.4	75.7	1.8	25.4	37.2	310	17	85	61.4	422

The statistics are presented using a bounded approach for the handling of non-detected/non-quantified data (therefore they are displayed as ranges). The upper bound of the range estimates the non-detected/non-quantified values using the reported limit of detection (LOD) or limit of quantification (LOQ) respectively. The lower bound of the range instead assumes the non-detected/non-quantified values as zero. When the lower bound and the upper bound of the range are coincident, only one number is presented. When the lower bound is zero, the range is represented by the upper bound prefixed by '<'. The table contains the number of samples (n), the mean and the 95-percentile (P95).

‘Beer’ and ‘wine, white, sparkling’ contains the higher mean values for the category beverages for the sum of biogenic amines (11.1 mg/kg and 12.1 – 12.4 mg/kg). For this class the major contributors to the sum were tyramine and putrescine.

For the category ‘condiments’ the difference in concentration values between ‘fish sauce’ (582 -588 mg/kg) and ‘other savoury sauces’ (24.2 – 56 mg/kg) is maintained as for the individual substances. All the amines equally contribute to the sum.

The category ‘fermented sausages’ (281 – 283 mg/kg) records higher values in the ‘meat products’. This food class is followed by ‘ripened meat products’ (100 – 101 mg/kg). For these classes, tyramine is the higher contributor to the sum.

‘Cheese’ records high values for the sum of biogenic amines. Overall this food class recorded mean values of 177 -334 mg/kg. ‘Acid curd cheese’ reaches the higher mean values (1460 mg/kg), however only four samples were available for this class. This subclass is followed by ‘washed rind cheese’ (220 - 388 mg/kg), blue cheese (188 -351 mg/kg), hard cheese (167 – 318 mg/kg) and fresh cheese (32.1 – 172 mg/kg). For sum concentrations for ‘cheese’, there was an equal contribution from tyramine and cadaverine followed by putrescine and histamine.

Also for ‘fermented vegetables’ the sum of biogenic amines reached high values (375 – 390 mg/kg). In this case, this value was mainly influenced by the contribution of putrescine (264 mg/kg).

In summary, the food categories showing the highest mean values for the sum of biogenic amines were ‘fish sauce’ (582 - 588 mg/kg), fermented vegetables (375 – 390 mg/kg ), ‘cheese’ (177 – 334 mg/kg) and ‘fermented sausages’ (281 – 283 mg/kg).

#### 4.1.3.8. Summary of occurrence data

Table 17 provides a summary of 95-percentiles (P95) statistics, calculated for each food category and for each biogenic amine. The P95 was used to represent high occurrence values. Higher percentiles were not considered reliable due to the limited number of samples available in some food categories as presented in Tables 10 – 15. For the selected occurrence values, the following remarks should be highlighted:

In the food category ‘fish and fish products’, ‘dried anchovies’ data are available only for histamine. All ‘cheese’ subclasses were aggregated, since P95 occurrence values were comparable, mitigating the impact of some subclasses such as ‘acid curd cheese’ where the number of samples was insufficient to have a reliable estimation of the statistics.

**Table 17:** Summary table of 95-percentile for biogenic amines expressed in mg/kg

Food class	Sub-category	Occurrence of biogenic amines (95-Percentile) mg/kg					
		Histamine	Tyramine	Putrescine	Cadaverine	Phenyl-ethylamine	Tryptamine
Alcoholic beverages	Beer	4.8	24.7	8.3	5.3	0.9	1.7
	Fortified and liqueur wines	2.8	21.3	3.6	0.3	-	-
	Wine, red	12.3 - 12.4	7.8 - 8.5	9.5 - 11.5	0.6 - 1.6	<5	-
	Wine, white	2.6	4.3 - 4.5	3.9 - 4.3	0.3 - 0.4	<1.5	-
	Wine, white, sparkling	5.2	26.4	15	0.2	-	-
Sauce	Fish sauce	597	421	167	502	58.6	244
	Other savoury sauces	<13.3	18.6	24.2	<20	<10	<10
Fish and fish products	Dried anchovies	1440	-	-	-	-	-
	Fermented fish meat	34.9	251	75.1	34.5	5.7 - 9.2	6.5
	Other fish and fish products	60.5 - 100	33.4	26.4	130	12.2	15.5
Meat products	Fermented sausages	149	397	334	154	34.7	42.9
	Other ripened meat products	35	149	136	84.1	3.3 - 10	<1
	Other meat products	4.8	67	123	25	1 - 1.1	1.1
Dairy products	Cheese	130	440	143	470	18.8	<50
	Yoghurt	1	5.2	1.1	10.3	-	-
	Other dairy products	0.6	0.4	0.9	3	-	-
Vegetables and vegetable products	Fermented vegetables	92	91	549	94	<5	-
	Other vegetables	<0.5	25.4	310	85	<9.3	-

The statistics are presented using a bounded approach for the handling of non-detected/non-quantified data, therefore they are displayed as ranges. The upper bound of the range estimates the non-detected/non-quantified values using the reported limit of detection (LOD) or limit of quantification (LOQ) respectively. The lower bound of the range instead assumes the non-detected/non-quantified values as zero. When the lower bound and the upper bound of the range are coincident, only one number is presented. When the lower bound is zero e.g. Tables 18 and 19 the range is represented by the upper bound prefixed by ‘<’. The table contains P95 statistics of occurrence values. (-) indicates not reported

#### 4.1.4. Consumption data

The EFSA Comprehensive European Food Consumption Database (Comprehensive Database) has been built from existing national information on food consumption at a detailed level. Competent organisations in the European Union’s Member States have provided EFSA with data from those most

recent national dietary surveys in their countries, at the level of consumption by the individual consumer. All food consumption data were codified according to the 'FoodEx classification system' which has been developed by the DATEX Unit in 2009 (EFSA, 2011a).

The Comprehensive Database includes methodological differences making these data not fully suitable for country-to-country comparisons, particularly for chronic long term exposure. Overall, the food consumption data gathered at EFSA in the Comprehensive Database are the most complete and detailed data currently available in the EU. Based on the detailed structure of the food classification (FoodEx) the "Comprehensive European Food Consumption Database" will allow intake estimation from more detailed food categories. The guidance for 'use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' describes the usage of this database for exposure assessment (EFSA, 2011b).

The exposure calculation performed for the exposure assessment of biogenic amines is an acute exposure. In such cases, the intake should be calculated on a meal or day basis. In the consumption database, the individual meals are recorded only for a few countries. Therefore, the exposure was calculated at a day level. The preferred option is therefore to use individual consuming days. Only the days where these food categories were consumed were considered. Consumption surveys spread from one up to seven days per individual. The usage of individual consuming days allows taking into account high consumption occurred by a subject in a specific day, if in the survey more days were recorded. Consuming days offer a conservative estimate of the exposure, since it will summate the contribution of more meals occurred on the same day. Table 18 shows the 95-percentiles (P95) of consuming days for the relevant food categories. The estimate of high values of consumption are provided using the 95-percentiles, since the P95 statistic is sufficiently safe to exclude outliers, which may be present in the food consumption surveys. In addition, it is necessary to split the consumption data by country and survey because of heterogeneity factors occurring during the consumption surveys. In fact, based on the calculation of high values of consumption, the table presents ranges, which describe the minimum and the maximum P95 calculated for each country and survey. In the Appendix the data for individual MSs are collated (Appendix B).

Looking at the consumption database it was not possible to find exact matching categories compared to these used in the occurrence part of this opinion (data are summarised in the chapter 4.1.3.7). The major issues were to identify "fermented" products, since this information is not explicitly considered in the FoodEx classification system. This information can only be deduced sometimes by the original food name. Therefore the following assumptions have been applied for in the different food categories:

- The category 'alcoholic beverage' has good consumption data coverage also at sub-category level.
- The category 'fish sauce' is not available in the consumption database. The FoodEx class 'soya sauce' was assumed to have a similar mode of use in the single meals and will thus be used for estimating these consumptions. Under this assumption, the other savoury sauces will not be considered for exposure, since the occurrence levels are lower than for 'fish sauce'.
- Consumption data for 'fermented fish' are not included in the database. Therefore the overall consumption for the general food category 'fish products' was used. As a consequence of this the exposure of the category 'fermented fish products' may be overestimated. The consumption of 'fish meat' has been used for 'other fish and fish products' since from the occurrence data, this category contains mainly fish meat. Data on 'dried anchovies' was also not available and given the specificity of this food, it will not be included in the exposure calculation.
- The class 'fermented sausage', containing higher occurrence values of biogenic amines in the entire category 'meat products' is linked with the FoodEx categories of 'semi-dried sausages' and 'dried sausages' available in the consumption database.

- In the category ‘dairy products’ the consumption data for ‘cheese’ and ‘yoghurt’ were available.
- The category ‘fermented vegetables’ was linked instead with the consumption of ‘vegetable products’, since information on consumption of fermented vegetables was rarely reported. The category ‘other vegetables and vegetable products’ containing mainly leafy vegetables and sprouts was linked with the FoodEx class ‘leafy vegetables’.

**Table 18:** Amounts of high food consumption in the EU20<sup>9</sup> (minimum and maximum 95-percentile (P95) of consumption days when the food was consumed).

Food category	Sub-category	High consumption amount (g/day; P95 min – P95 max)
Alcoholic beverages	Beer	750 - 5040
	Fortified and liqueur wines	50 - 400
	Wine, red	200 - 1000
	Wine, white	50 - 1500
	Wine, white, sparkling	250 - 740
Savoury sauces	Fish sauce	0.7 – 50
Fish and fish products	Fermented fish products	8 – 360
	Other fish and fish products	145 – 414
Meat products	Fermented sausages	43.4 – 250
	Other ripened meat products	39 – 284
	Other meat products	170 – 300
Dairy products	Cheese	100 – 247
	Yoghurt	250 – 796
	Other dairy products	300 – 954
Vegetables and vegetable products	Fermented vegetables	8.9 – 300
	Other vegetables	43.2 – 302

Amounts of food eaten by high consumers in one day, extracted from the “Comprehensive European Food Consumption Database”. The amounts are expressed in gram/day. The statistics reflects only the adult population and the actual days of consumption for the food categories presented. The 95-percentile statistics of the consuming days was used to quantify the high consumption amounts. The range represents the minimum and maximum P95 consumption (g/day) of each food categories between countries

#### 4.1.5. Exposure assessment

The first objective of the exposure analysis was to calculate the exposure for the biogenic amines in all identified food categories and to conclude on the food categories which pose a major risk for consumers.

The amount of food representing the majority of consumers for each food category of interest was extracted from the consumption database in relation to P95, as presented in 4.2. These data will be combined with the occurrence data described in 4.1.3.7. For the occurrence data, the P95 statistics for the concentration of biogenic amines for each food category of interest was used to estimate values of

<sup>9</sup> Consumption data at countries and surveys level is reported in Appendix B.

occurrence for foods containing high amounts of biogenic amines. The combination of these data is provided in Table 19, showing high exposure values for the food categories of interest. The exposure values are provided in mg/day. The ranges shown reflect the combination of the lower bound of occurrence and of the upper bound of occurrence multiplied respectively with the ranges of P95 consumption presented in Table 18.

For histamine, the highest exposure values were calculated for 'other fish and fish products' (8.8 – 41.4 mg/day), followed by 'fermented sausages' (6.4 – 37.1 mg/day), 'cheese' (13 – 32.1 mg/day) and 'fish sauces' (0.4 – 29.9 mg/day).

For tyramine, the class with highest values were calculated for 'beer' (18.5 – 124.6 mg/day). The reason can be mainly attributed to the occasional high values recorded in the consumption database. 'Cheese' also shows high exposure (44 – 108.7 mg/day) to this biogenic amine. 'Fermented sausages' (17.2 – 99.3 mg/day) and 'fermented fish meat' (2 – 90.4 mg/day) can lead to very high exposure to this biogenic amine.

For putrescine, the food categories with the highest exposures are different from those for the other biogenic amines and are 'fermented vegetables' (4.9 – 164.7 mg/day) and 'other vegetables' (13.4 – 93.6 mg/day). After vegetables, the category providing the highest exposure values is 'fermented sausages' (14.5 – 83.6 mg/day), 'other meat products' (20.9 – 36.9 mg/day) and 'cheese' (14.3 – 35.3 mg/day) follows with relative lower values.

For cadaverine, 'cheese' is the top contributor with 47 – 116.1 mg/day followed by the category 'other fish and fish products' (18.9 – 53.8 mg/day).

For phenylethylamine and tryptamine, occurrence data were not available for all the categories of interest. It can, however, be concluded that the exposure levels are lower than for other biogenic amines.

For phenylethylamine the higher exposure levels were calculated for 'fermented sausages' (1.5 – 8.7 mg/day) and 'cheese' (1.9 – 4.6 mg/day).

For tryptamine, the highest exposure levels are calculated for 'cheese' (0.1 – 12.3), 'fish sauce' (0.2 – 12.2 mg/day) and 'fermented sausages' (1.9 – 10.7 mg/day).



**Table 19:** Biogenic amines high exposure values per day of consumption of relevant food

Biogenic amines high exposure for relevant food categories (mg/day)							
		Histamine	Tyramine	Putrescine	Cadaverine	Phenylethylamine	Tryptamine
Alcoholic beverages	Beer	3.6 - 24.2	18.5 - 124.6	6.2 - 41.9	4 - 26.7	0.6 - 4.3	1.3 - 8.6
	Fortified and liqueur wines	0.1 - 1.1	1.1 - 8.5	0.2 - 1.4	<0.1 - 0.1	-	-
	Wine, red	2.5 - 12.4	1.6 - 8.5	1.9 - 11.5	0.1 - 1.6	<0.1 - 5	-
	Wine, white	0.1 - 3.9	0.2 - 6.8	0.2 - 6.5	<0.1 - 0.6	<0.1 - 2.3	-
	Wine, white, sparkling	1.3 - 3.8	6.6 - 19.5	3.8 - 11.1	0.1	-	-
Fish and fish products	Fermented fish meat	0.3 - 12.6	2 - 90.4	0.6 - 27	0.3 - 12.4	<0.1 - 3.3	0.1 - 2.3
	Other fish and fish products	8.8 - 41.4	4.8 - 13.8	3.8 - 10.9	18.9 - 53.8	1.8 - 5	2.2 - 6.4
Meat products	Fermented sausages	6.4 - 37.1	17.2 - 99.3	14.5 - 83.6	6.7 - 38.5	1.5 - 8.7	1.9 - 10.7
	Other ripened meat products	1.4 - 9.9	5.8 - 42.1	5.3 - 38.6	3.3 - 23.9	0.1 - 2.8	<0.1 - 0.3
	Other meat products	0.8 - 1.4	11.4 - 20.1	20.9 - 36.9	4.3 - 7.5	0.2 - 0.3	0.2 - 0.3
Dairy products	Cheese	13 - 32.1	44 - 108.7	14.3 - 35.3	47 - 116.1	1.9 - 4.6	0 - 12.3
	Yoghurt	0.3 - 0.8	1.3 - 4.1	0.3 - 0.9	2.6 - 8.2	-	-
	Other dairy products	0.2 - 0.6	0.1 - 0.4	0.3 - 0.9	0.9 - 2.9	-	-
Savoury sauces	Fish sauce	0.4 - 29.9	0.3 - 21	0.1 - 8.3	0.4 - 25.1	<0.1 - 2.9	0.2 - 12.2
Vegetables and vegetable products	Fermented vegetables	0.8 - 27.6	0.8 - 27.3	4.9 - 164.7	0.8 - 28.2	<0.1 - 1.5	-
	Other vegetables	<0.1 - 0.2	1.1 - 7.6	13.4 - 93.6	3.7 - 25.6	<0.1 - 2.8	-

Exposure calculation in the food categories of interest considering high amounts of biogenic amines (P95 occurrence) and high consumption of food (P95 of consumption days). The results are provided in mg/day. When the lower bound and the upper bound of the range are coincident, only one number is presented. When numbers were below 0.1 they are reported using the sign below (“<”) as “<0.1”. ( - ) not reported

The second objective of the exposure analysis was to calculate an overall exposure per day to biogenic amines from the relevant food categories. It should be pointed out that a complete one day cumulative exposure cannot be calculated, since the available occurrence data cover only part of the diet. However, a cumulative exposure of mg per day, related to the food categories of interest can be calculated. This may represent an estimate of the total cumulative exposure per day only if the contributions of the other food categories, for which occurrence data are not available, are negligible.

As anticipated, the exposure calculations performed in Table 19 are high exposure values for each food category reported in the table. The calculation of a cumulative exposure per day as the sum of the figures reported in this table would represent an unrealistic overestimation, since high consumption levels of the food categories by the same person/consumer are not independent but correlated; in fact it is unlikely that a person would be consuming high amounts of all food categories in the same day.

Therefore, the calculation of the cumulative exposure per day (Table 20) was performed taking into account the combinations of foods eaten on the same day by the same consumer, based on data available in the consumption database at an individual level. The exposure to highly contaminated food (4.1.3.8) was combined for each day of consumption, for all subjects available in the consumption database. The 95-percentile statistics was used to represent high levels of exposure.

It is important to note that, due to the assumptions made for matching the occurrence data with the consumption data and the use of all consuming days, the statistics provided are only an estimation of an high exposure scenario and do not reflect a specific percentile of the actual population. In addition, values in Table 20 may be higher than values for cumulative exposures, since those consuming high amounts of food in one category do not automatically match with high consumption of food combinations.

**Table 20:** One day cumulative exposure (P95) of all considered food categories calculated for consuming day.

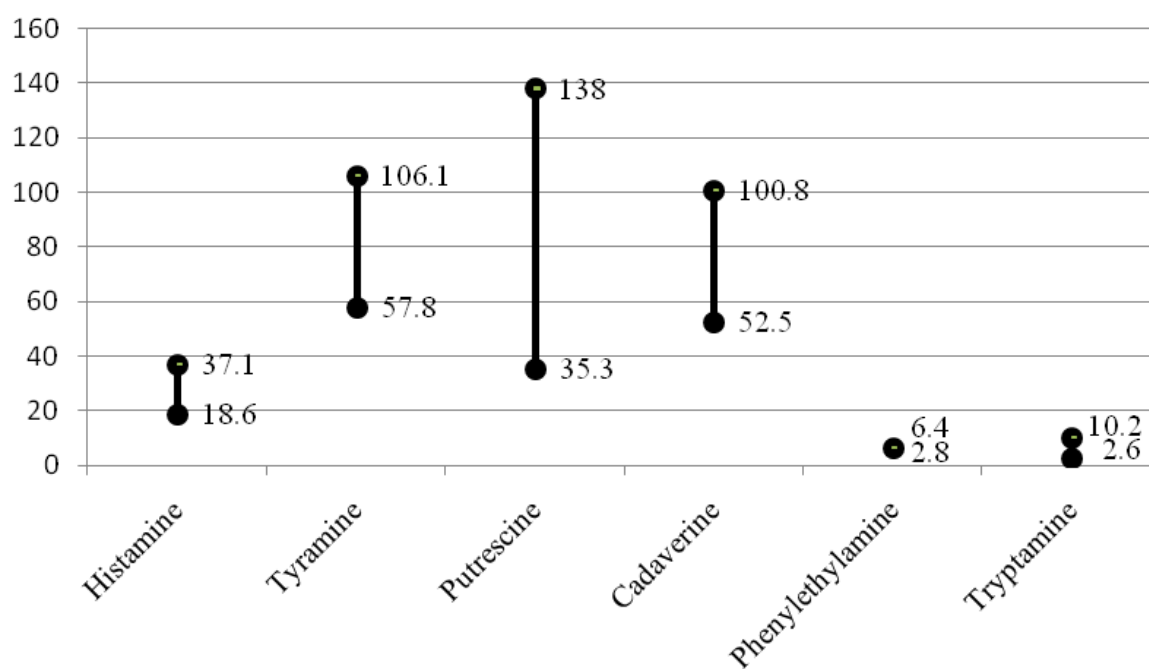
Cumulative exposure for relevant food categories by country/survey (mg/day)						
Country	Histamine	Tyramine	Putrescine	Cadaverine	Phenylethylamine	Tryptamine
Austria	30.3 - 32.3	88.7 - 88.8	78	80.3 - 80.5	4.9 - 5.7	4.7 - 9.3
Belgium	29.4 - 32.7	89.9 - 90	60.7 - 61	87.4 - 87.5	4.6 - 5.7	3.6 - 9.7
Bulgaria	23.4 - 28.2	68.3	75.3 - 75.4	70.5	3.8 - 4.6	3.7 - 7.5
Czech Republic	34.2 - 37	106	108	78.1	5.3 - 6.2	6.3 - 9.8
Denmark	21.7 - 24.1	67.8	40.3 - 40.6	57.4 - 57.5	3.2 - 4.6	3.1 - 6.6
Estonia	28 - 30.8	91.6	53.6	88.4	4 - 4.7	3.5 - 9.8
Finland	26.9 - 30.4	77.3 - 77.5	67.2	73 - 73.2	3.8 - 4.6	3.5 - 7.9
France	30 - 32.9	88.9 - 89	68.6 - 68.8	93.4	4.3 - 5.6	2.9 - 9.6
Germany	29.8 - 32.1	90.9	91.2 - 91.3	82.8 - 82.9	4.3 - 5.4	3.9 - 8.9
Hungary	30.7 - 32	72.7	138	64.8	3.9 - 4.4	4.3 - 7.1
Ireland	23.7 - 27.7	82.2	47.3 - 47.4	53	3.7 - 4.6	5.2 - 7
Italy	31 - 36.7	91.6 - 91.7	80.6 - 80.9	101	4.9 - 6.4	3.8 - 10.2
Latvia	25.6 - 26.3	77.9 - 78.1	46	72.1	3.9 - 4.2	3.7 - 8
Netherlands	28.5 - 29.1	94.1	72	75.5	4 - 4.9	4.2 - 9.3
Poland	33.5 - 37.1	88	128	90.5 - 90.7	3.9 - 4.6	2.6 - 8.9
Slovakia	29.8 - 30.1	84.1	97.2	57.4	5.2 - 5.6	6 - 8.4
Slovenia	23.3 - 23.4	60	90.5 - 90.7	57	3.5 - 4.4	2.8 - 5.8
Spain survey I AESAN	28.2 - 34.2	75.7 - 75.8	68.9	76.8	4.9 - 5.8	5.1 - 8.7
Spain survey II AESAN FIAB	27.4 - 36.2	64.6	67.7 - 67.8	71.8 - 71.9	4.9 - 6.2	5.3 - 7.7
Sweden	18.6 - 22.6	57.8 - 57.9	35.3 - 35.4	52.5 - 52.7	2.8 - 3.7	2.7 - 5.6
United Kingdom	22.5 - 26.5	70.9 - 71	47.9 - 48.1	55.1 - 55.3	3.3 - 4.3	4.1 - 6.6

When the lower bound and the upper bound of the range are coincident, only one number is presented.

As already performed for the exposure calculation at food category level, to highlight eventual differences between the consumption behaviours in different countries and to highlight potential influences of the consumption survey design, the 95-percentile of cumulative exposure was calculated for each country and survey separately.

The variability in the ranges of Table 20 represents the difference between lower bound and upper bound in the occurrence data. It is of note that overall, this difference is not broad.

Calculating the minimum lower bound and maximum upper bound for each biogenic amine in Table 20, Figure 3 can be derived, showing a summary of the partial cumulative exposure to each biogenic amine.



**Figure 3:** Summary of cumulative exposure P95 range between the countries for biogenic amines presented of all considered food categories.

As anticipated, summary exposure values presented in Figure 3 and Table 19 reflect P95 of biogenic amine occurrence combined with high food consumption data (P95 of one day consumption). The cases of intoxication reported in the literature and in the RASFF database are due to a single food consumption event and in some cases exceeding the P95 of occurrence used for the exposure calculation. It is important therefore to calculate concentration levels of biogenic amines resulting in an exposure from a single food consumption within the toxicological threshold. An approach for this calculation is to use the BA thresholds - histamine (50 mg for healthy individuals; section 3.1.3.3.) or tyramine (600 mg for healthy individuals and 50 mg for individual under RIMA medications; 3.2.3.1.) - and the high values for the consumption of the relevant food categories in Table 18.

These results are presented in the Table 21. It is of note that the levels calculated for tyramine are higher than the occurrence data received, in fact no sample exceeded these levels. For histamine there is small percentage of samples exceeding these indicative levels. The categories where this

exceedence is more evident are ‘other fish and fish products’ (3.7% samples), ‘cheese’ (3.0% samples), ‘beer’ (2.1 %) and ‘other ripened meat products’ (2.1%).

**Table 21:** Percentage of mainly fermented food concentrations of concern calculated from BA threshold estimates (sections 3.1.3.3. and 3.2.3.1) and consumption data (Table 18).

Food category	Sub-category	Histamine		Tyramine		Sensitive population under RIMA	
		Concentration based on estimated threshold (mg/kg of food)	% samples exceeding calculated concentration	Concentration based estimated threshold (mg/kg of food)	% samples exceeding calculated concentration	Concentration based on estimated threshold (mg/kg of food)	% samples exceeding calculated concentration
Alcoholic beverages	Beer	10	2.1	119	-	10	13
	Fortified and liqueur wines	125	-	1500	-	125	-
	Wine, red	50	-	600	-	50	-
	Wine, white	33	0.4	400	-	33	-
	Wine, white, sparkling	68	-	811	-	68	-
Savoury sauces	Fish sauce	1000	-	12000	-	1000	-
Fish and fish products	Fermented fish products	139	1.4	1667	-	139	7.1
	Other fish and fish products	121	3.7	1449	-	121	1.2
Meat products	Fermented sausages	200	-	2400	-	200	24
	Other ripened meat products	176	2.1	2113	-	176	3.3
	Other meat products	167	1.3	2000	-	167	1.3
Dairy products	Cheese	202	3.0	2429	-	202	11
	Yoghurt	63	-	754	-	63	-
	Other dairy products	52	-	629	-	52	-
Vegetables and vegetable products	Fermented vegetables	167	-	2000	-	167	-
	Other vegetables	166	-	1987	-	166	-

( - ) not reported

Sensitive consumers taking RIMA drugs (estimated threshold of 50 mg) may tolerate some tyramine containing food (Table 21). From the data received the consumption of ‘fermented sausages’, ‘fermented fish products’ and ‘cheese’ appear to be more likely to exceed the estimated threshold of 50 mg. The high consumption of ‘beer’ will also allow the exceeding of this estimated threshold. Since this sensitive population is aware of constraints on consumption of food, some consideration of ‘moderate’ consumption can be done. In fact, considering a moderate consumption of beer of 500 ml per meal, the calculated concentration in the food becomes 100 mg/kg, therefore the estimated threshold of 50 mg is not exceeded by any of the samples collected. In the same way, moderate consumption of around 130 g of other ripened meat products, where the maximum tyramine concentration in the data is 387 mg/kg, would result in an exposure to tyramine within the estimated threshold. For ‘fresh cheese’, which has a maximum concentration of 457 mg/kg, a portion of 110 g would reach the estimated threshold. The same consideration of reducing the portion size can be applied also to the other foods, although it may result in considering too small amounts consumed. It is important to keep in mind that these considerations were done on the basis of the consumption of each food taken on its own.

For most sensitive consumers taking classical MAOI drugs (estimated threshold level 6 mg) such calculation can not be reliably done due to the low threshold and the uncertainties linked to the tyramine occurrence and food consumption data. Nevertheless, from the occurrence data (Table 11) it

is clear that the fermented foods are of particular concern for these individuals, which is consistent with advice given with medical prescription of classical MAOI drugs to follow a 'tyramine free diet' (Mariné-Font et al., 1995).

## **4.2. Uncertainty**

The evaluation of the inherent uncertainties in the assessment of exposure to biogenic amines has been performed following the guidance of the Opinion of the Scientific Committee related to Uncertainties in Dietary Exposure Assessment (EFSA, 2006). In addition, the report on "Characterizing and Communicating Uncertainty in Exposure Assessment" has been considered (WHO/IPCS, 2008). According to the guidance provided by the EFSA opinion (EFSA, 2006) the following sources of uncertainties have been considered: assessment objectives, exposure scenario, exposure model, and model input (parameters).

### **4.2.1. Assessment objectives**

The objectives of the assessment were clearly specified in the terms of reference. The BIOHAZ Panel assessed the occurrence data that were collected by EFSA, and carried out an exposure assessment for the general adult population. The uncertainty in the assessment objectives is considered to be negligible.

### **4.2.2. Exposure scenarios/Exposure model**

In response to EFSA's request to submit biogenic amines occurrence data in food, nine European countries submitted data and a different number of samples was available for the different biogenic amines. For histamine, EFSA received data for 10123 samples while for phenylethylamine only data for 1235 samples were submitted. The food products for which data were provided varied between submissions from the different European countries, but most samples belonged to the 'fish and fish product' category, followed by 'cheese' and 'meat products'. Most of the providers did not submit a complete coverage of the categories of interest, but in most cases they covered only some of these classes. Moreover, there are differences in the number of biogenic amines reported. The majority of the analyses were for histamine in fish and fish products.

Data for a complete coverage of the diet was not available, therefore it was not possible to perform a complete exposure but the exposure was calculated in reference to the food categories of interest for which data was available. In any case, it is assumed that the food categories presented cover most of the exposure. The presence of low level of preformed BAs in some food classes not included in the exposure assessment such as fruits and fruit juices should not impact on the overall exposure particularly for histamine and tyramine which provide the major source of risk.

There is uncertainty in possible regional differences in biogenic amines contamination of food commodities, particularly due to the differences in food processing in the different regions. In addition, due to the presence of targeted data coming from control and monitoring programs particularly on fish and fish products, the data should be considered not fully representative of food on the EU market. The presence of targeted data would lead to overestimate exposure.

The high proportion of samples having levels below the LOD or LOQ may have introduced uncertainties to the overall estimate. The use of the upper bound, for risk characterisation in this opinion tends to overestimate the dietary exposure. The usage of P95 of the occurrence concentration combined the P95 of one day consumption by food consumers of the categories of interest should also provide an overestimate of the one meal exposure to BAs which was the target of the exercise. Uncertainty also originates from the fact that some consumption food groups were not available and

some assumptions were made to match occurrence figures. Mainly, more frequently consumed categories were used which should lead to overestimation of the exposure.

#### 4.2.3. Model input (parameters)

As outlined in section 7.2.2.2, the HPLC method is cited as the reference method for histamine in European Commission Regulation (EC) No 2073/2005 (microbiological criteria for foodstuffs) for fishery products, however there is considerable methodological variation likely to be present for analysis of histamine in all other foods, in addition to other BAs where there are no prescribed fixed official methods provided it can be demonstrated in a traceable manner that they fulfil the requirements according to European Regulation (EC) No 882/2004 for Official Laboratories to operate and be accredited in accordance with the relevant Standards (e.g. ISO/IEC 17025). Inter-laboratory methodological differences for the analysis of BAs may have contributed to the uncertainty in the analytical results. The lack of certified reference materials, and suitable external quality assessment schemes is a further limitation in assessment of the overall uncertainty in the analytical results

Vidal-Carou et al. (2009) compared the accuracy (percentage recovery) and precision (percentage relative standard deviation (RSD)) for chromatographic procedures applied to meat and meat products. Accuracy for histamine and tryptamine varied between 76->100% and for putrescine, cadaverine, phenylethylamine and tryptamine 82->100%. For precision histamine and tryptamine varied between 2.5-10.8% and for putrescine, cadaverine, phenylethylamine and tryptamine 2.6 -18%. Few studies have compared the performance and the concordance between analytical methods for biogenic amines in meat and meat products. In an examination of Czech dry fermented sausages, HPLC procedures after precolumn derivatization of dansyl chloride and *o*-phthalaldehyde gave similar results in terms of detection limit, repeatability, recovery, and accuracy (Smela et al., 2004). However, these authors reported that *o*-phthalaldehyde derivatization was faster and much simpler in terms of sample pretreatment, which can be fully automated by the auto-sampler. In another study (Ansorena et al., 2002), the application of modifications of DnCl-based methodologies by three laboratories significantly affected the results obtained on biogenic amine accumulation in European fermented sausages. Two laboratories used 0.4 M perchloric acid as the extractant and 1,7-diaminoheptane as the internal standard, the derivatization was carried out for 40 min, after which the sample was dissolved in acetonitrile (Eerola et al., 1993). The third laboratory used acetone and 5% trichloroacetic acid as extractant solvent and 1,8-diaminooctane as internal standard. The derivatization was performed for a longer period (4 h), followed by a further extraction of the amines with diethyl ether before the sample was dissolved in acetonitrile. The amines most affected by the method of analysis were tryptamine and tyramine (Ansorena et al., 2002).

#### 4.2.4. Other uncertainties

Overall, the sensitivity against biogenic amines is increased due to a weakened enzymatic amine degradation caused by genetic, physiological or acquired impairment of MAO, DAO, HNMT function (see section 3.4).

For histamine, some toxicological data are available from experiments with healthy volunteers and sensitive individuals. These studies do not provide the body weights of the test persons. Furthermore the results are not always reproducible due to the intra- and interindividual variations in sensitivity. Nevertheless, the studies give some indication for dose-response-relationship and a toxicological threshold level as an acute reference dose can be estimated. Data from outbreaks show big variation in concentrations leading to adverse effects in the consumer. Variations in sensitivity may also be the result of interaction with other biogenic amines, other diet constituents such as alcohol or medication with DAO inhibitors. These uncertainties may lead either to an underestimation of the adverse effect to occur in sensitive people or to an overestimation of the risk in healthy people.



For tyramine, toxicological data is derived from some clinical studies dealing with the interaction between dietary tyramine and MAOI drugs (see section 3.2.3.1). These studies are usually performed in healthy volunteers without MAOI medication (i.e. placebo group). The results allow the amount of tyramine needed to provoke a clinically significant increase of systolic blood pressure, of at least 30 mmHg (PD30). Besides inter- and intraindividual variability, the sensitivity to oral tyramine may also vary depending on the diet composition. Lipids but also proteins seem to significantly reduce the tyramine bioavailability (Audebert et al., 1992), though other substances such as alcohol or other biogenic amines can increase tyramine absorption and thus altering/enhancing its vasopressor effect. This uncertainty leads to both underestimation of the adverse vasopressor effect or to an overestimation of the risk.

For putrescine and cadaverine, only a few *in vitro* and *in vivo* experimental data are available about their potentiating effect on the toxicity of histamine and tyramine. Also some data from experiments with humans are available. However no dose-response relationship could be determined and no conclusions could be drawn from these experiments. Therefore, no assessment about their potentiating effect on the toxicity of histamine and tyramine can be conducted. This uncertainty may lead to an underestimation of the risk due to biogenic amines, since only the risk assessment of the individual biogenic amines is feasible at the moment.

#### 4.2.5. Summary of uncertainties

In Table 22 a summary of the uncertainty evaluation is presented, highlighting the main sources of uncertainty and indicating an estimate whether the respective source of uncertainty might have led to an over- or underestimation of the exposure or the resulting risk.

**Table 22:** Summary of qualitative evaluation of the impact of uncertainties on the risk assessment of the dietary exposure to biogenic amines.

Sources of uncertainty	Direction
Measurement uncertainty of analytical results	+/- <sup>(a)</sup>
Limited number of food categories and availability of food consumption data	+/-
Extrapolation of occurrence data from a limited countries to whole Europe	+/-
Influence of upper-bounds for non-detects on exposure estimate	+
Lack of information on the impact of food processing and storage	+/-
Limited information on the dose-response-relationship for histamine and tyramine to cause adverse health effects	+/-
Limited information on the toxicity of putrescine and cadaverine to potentiate adverse health effects of other biogenic amines e.g. histamine and tyramine	-

(a): + indicates uncertainty with potential to cause over-estimation of exposure/risk; - indicates uncertainty with potential to cause under-estimation of exposure/risk.

The impact of the uncertainties on the risk assessment of exposure to biogenic amines is considerable. These uncertainties may lead either to an underestimation of the adverse effect to occur in sensitive people or to an overestimation of the risk in healthy people. However, the approach used including high percentiles (P95) should reasonably provide an overestimation and counter balances potential underestimates.

## 5. Qualitative risk characterisation related to fermented foods

Risk ranking has been performed in two steps: first according to the occurrence levels and then through consumption of the most relevant foods.

### 5.1. Healthy individuals

Overall, healthy individuals can detoxify dietary biogenic amines by acetylation and oxidation reactions mediated by the enzymes, MAO, DAO and HNMT.

However, very high amounts of biogenic amines ingested with food may present a risk to all individuals (Taylor, 1986; Bardocz, 1995). Toxic effects of biogenic amines can develop through both increased availability (due to high amounts ingested with food) and/or impaired biogenic amine degradation (due to increased sensitivity of individuals).

According to the dose-response relationship estimated in experiments with healthy volunteers, no toxicologic effects were reported for histamine up to 50 mg histamine per person. The ingestion of amounts of 75 mg histamine and above may cause symptoms of flushing, diarrhoea and headache. In relation to histamine fish poisoning data, fish containing less than 50 mg/kg seems to be safe for human consumption, whereas concentrations between 50 and 200 mg/kg may cause adverse health effects and levels above 200 mg/kg histamine are reported to cause toxic effects in humans.

According to the literature (section 3.2.3.1) from 600 mg up to 2000 mg of tyramine administered in a meal would be needed to cause a minimal pressure increase (of at least 30 mmHg) (Korn et al., 1988a,b; Berlin et al., 1989; Zimmer et al., 1990; Patat et al., 1995). A dose-response curve was drawn by Patat et al. (1995), in which 1100 mg of tyramine corresponded to the effective dose (ED<sub>50</sub>) as the tyramine dose at which 50% of the individuals responded.

### 5.2. Individuals with increased sensitivity

For patients with histamine intolerance and chronic headache, a histamine-free diet is the treatment of choice, because even small amounts of histamine may cause adverse health effects (Jarisch, 2004).

A recent critical review regarding health effects for tyramine in food was carried out by McCabe-Sellers et al. (2006) which concluded that the presence of 6 mg in one or two usual servings is thought to be sufficient to cause a mild adverse event while 10–25 mg will produce severe adverse effects in patients treated with classical MAOI treatment (McCabe, 1986). Other reports conclude that from 50 up to 150 mg of tyramine would be well tolerated by patients under new generation MAOI treatment, so called RIMA (reversible inhibitors of MAO-A) (Table 4; Korn et al., 1988a,b; Dingemanse et al., 1998; Patat et al., 1995).

### 5.3. Risk ranking

#### 5.3.1. Histamine

According to occurrence data presented in Table 10, the food categories showing the highest mean values of histamine are ‘dried anchovies’ (348 mg/kg), ‘fish sauce’ (196–197 mg/kg), ‘fermented vegetables’ (39.4 – 42.6 mg/kg), ‘cheese’ (20.9 – 62 mg/kg), ‘other fish and fish products’ (26.8 – 31.2 mg/kg) and ‘fermented sausages’ (23.0 – 23.6 mg/kg).

For histamine, the high exposure values were calculated for ‘other fish and fish products’ (8.8 – 41.4 mg/day) followed by ‘fermented sausages’ (6.4 – 37.1 mg/day), ‘cheese’ (13 – 32.1 mg/day) and ‘fish sauces’ (0.4 – 29.9 mg/day).

**Table 23:** Comparison of histamine intake levels from the five most relevant food categories with the estimated toxicological threshold of 50 mg per person

Food category	Histamine intake (mg/kg)	Percentage of threshold (%)
Other fish and fish products	41.4	83
Fermented sausages	37.1	74
Cheese	32.1	64
Fish sauce	29.9	60
Fermented vegetables	27.6	55

The individual intake of the main food categories that contribute to histamine intake does not exceed the toxicological threshold of 50 mg. The food category being closest to the threshold is ‘other fish and fish products’ with an exhaustion of the threshold up to 83%. Consumption of ‘fermented sausages’, ‘cheese’, ‘fish sauce’ and ‘fermented vegetables’ leads to an exhaustion of the threshold of 74%, 64%, 60% and 55%, respectively (Table 23). Nevertheless, the threshold may be exceeded by the intake of more than one food item containing high amounts of histamine during the same meal.

### 5.3.2. Tyramine

According to the occurrence values presented in Table 24, the food categories showing the highest mean values of tyramine were ‘fermented sausages’ (136 mg/kg), ‘fish sauce’ (105 -107 mg/kg), ‘cheese’ (68.5 - 104 mg/kg), ‘fermented fish’ (47.2 -47.9 mg/kg) and ‘fermented vegetables’ (45 – 47.4 mg/kg).

For tyramine the class with high exposure values were calculated for ‘beer’ (18.5 – 124.6 mg/day). ‘Cheese’ also showed high exposure (44 – 108.7 mg/day) for this biogenic amine. ‘Fermented sausages’ (17.2 – 99.3 mg/day) and ‘fermented fish meat’ (2 – 90.4 mg/day) can lead to very high exposure to this biogenic amine.

**Table 24:** Comparison of tyramine intake levels from the five most relevant food categories with the estimated toxicological thresholds

Food category	Tyramine intake (mg/kg)	Percentage of threshold*	
		(for 600 mg threshold)	(for 50 mg threshold)
Beer	124.6	20.8	249.2
Cheese	108.7	18.1	217.4
Fermented sausages	99.3	16.6	198.6
Fermented fish meat	90.4	15.1	180.8
Other ripened meat products	42.1	7.0	84.2

\*600 mg for healthy individuals; 50 mg for patients taking RIMA drugs

The individual intake of the main food categories that contribute to tyramine intake does not exceed the toxicological threshold of 600 mg. Even if the five main sources were combined at the same meal, their contribution would be of 77% of the 600 mg tyramine threshold (Table 24).

The high exposure values estimated for beer, cheese, fermented sausages and fermented meat fish substantially exceed (from 180.8 to 249.2%) the 50 mg threshold for individuals taking reversible and selective (3<sup>rd</sup> generation) MAOI drugs.

For highly sensitive consumers, i.e. those under classical MAOI medication, the estimated threshold level 6 mg is easily reached by the consumption of fermented foods. Therefore, these sensitive individuals should avoid all tyramine containing food.

## 6. Control options

Biogenic amine in food are mainly produced by amino acid decarboxylase-positive microorganisms, therefore biogenic amines can accumulate in a wide variety of foods and beverages that have allowed the microbial growth and activity during its manufacture, handling and/or storage. Efforts to reduce the occurrence of biogenic amines in food deserve a priority and justify the challenge to the food industry to provide products with biogenic amine levels as low as possible.

The total contents of the different amines strongly depend upon the nature of the product, the microorganisms that are present and the environmental conditions. Overall, aminogenesis processes depend on multiple and complex variables, all of which interact, making it difficult to characterize the effects of each technological factor on aminogenesis during food fermentation, ripening and/or storage. Although the influence of these environmental factors is not always well characterised, they constitute the basis of the mitigation options to prevent or limit biogenic amine accumulation in foods.

Overall the current control of biogenic amine accumulation in food and beverages is based on two approaches:

1. Assurance and maintenance of **hygienic quality** of raw materials and production processes in order to limit the contamination of fermented food products with aminogenic microorganisms.
2. Implementation of specific **conditions and/or production techniques** aiming to:
  - 2a. Inhibit (eliminate) microorganisms with aminogenic potential,
  - 2b. Prevent the growth and minimize the decarboxylase activity of microorganisms.

In the literature, attempts to destroy biogenic amines have been described. However they are not according to general principles of food hygiene which relay on prevention rather than eliminating problems after they appeared; they have not been proven as effective and feasible.

The current control options to minimise biogenic amine occurrence in foods are mainly focussed on the food processing level, including raw materials handling and the fermentation process, as they constitute the most important factors for the biogenic amine accumulation in fermented products. During the storage of fermented food, the risk of biogenic amine accumulation is less important and is dependent on the above mentioned previous stages.

### 6.1. Raw material

In raw materials (i.e. fresh commodities), biogenic amine production generally results as a consequence of product mishandling. Therefore, aminogenesis should be prevented by improving food handling standards through a preventative strategy from harvest/slaughtering to the consumer. To this respect, food quality and safety management relying on HACCP should be regarded as the primary approach (Hungerford, 2010).

Within the HACCP process, good hygienic and manufacturing practices (GHP/GMP) along with proper cleaning and disinfection procedures should be carefully implemented from primary production. Aminogenic organisms originating from animals (i.e. intestines, skin, fish gills, etc) can be spread to other sites, surfaces and equipments during handling of fresh raw materials (such as

during degutting and filleting of fish, slaughtering, cutting and mincing of meat) and consequently promote the accumulation of biogenic amines during further processing (i.e. fermentation) and/or storage. Furthermore, any additional source of aminogenic microorganisms, decarboxylase enzymes or preformed biogenic amines should be minimised, for instance assuring an optimal quality of water, brine for salting, raw materials as well as of spices and ingredients used during product manufacturing (Innocente et al., 2009, Latorre-Moratalla et al., 2007).

The routes of contamination are not well identified, which makes the implementation of efficient measures difficult to avoid contamination of raw material. To control biogenic amine accumulation, the hygiene of raw materials should be improved and optimised through the reduction of the levels of microbial contamination. Intervention strategies to improve the hygiene of raw materials should include, whenever possible, thermal treatments. Clearly, pasteurisation of milk for cheese manufacture is commonly applied in the dairy industry and has proved to limit biogenic amine accumulation in comparison to production of cheese from raw milk (Novella-Rodríguez et al., 2004,a,b Ordóñez et al., 1997, Schneller et al., 1997). For certain commodities (fish and meat) thermal treatments are not possible due to detrimental changes to raw materials. In these situations, alternative non-thermal technologies, such as irradiation and high hydrostatic pressure, offer a potential as shown in studies dealing with fish, meat and milk hygiene (Latorre-Moratalla et al., 2007, Novella-Rodríguez et al., 2002, Ruiz-Capillas and Jimenez Colmenero, 2010, Vidal-Carou et al., 2007). However, these measures deserve further research to be applied to the industrial food chain. Freezing of meat and fish cannot be considered as a measure of microbial inactivation, although several reports show that when thawed, frozen meat and fish are less susceptible to biogenic amine accumulation than unfrozen counterparts, probably because the microbiota are reduced to some extent as a result of the freezing process (Bover-Cid et al., 2006a, Flick and Granata, 2005).

Attempts to eliminate biogenic amines once formed in raw materials have been undertaken, for instance by the use of amine-oxidase positive organisms (Gardini et al., 2002, Leuschner and Hammes, 1998b) or by gamma-irradiation (Kim et al., 2004). However, the effectiveness of these procedures has not been fully demonstrated in industrial food production systems. Alternatively, they may be controversial because elimination of biogenic amines could mask improper hygienic and manufacturing practices (Vidal-Carou et al., 2007).

It is generally accepted now that the reduction of growth of strong amine-producing bacteria through the optimisation of handling, processing and storage conditions and processing are the most effective methods (Dalgaard et al., 2008, Ruiz-Capillas and Jimenez Colmenero, 2010, Vidal-Carou et al., 2007).

The time/temperature binomial is the most important risk factor for the formation of histamine and other biogenic amines during handling and storage of fresh commodities (e.g. meat and seafood products). For this reason, low temperatures should be applied during storage to inhibit proteolytic and decarboxylase activity of bacteria (Hernández-Orte et al., 2008; Rezaei et al., 2007). However, proteolysis inhibition is not applicable for cheeses or fermented sausage manufacture because it is an essential process for coagulation and ripening.

Apart from mesophilic aminogenic organism which can be controlled by preventing time-temperature abuse, psychrotolerant bacteria are also relevant in relation to biogenic amine production in fish stored at chill temperature (Emborg and Dalgaard, 2006, Emborg et al., 2006). Freezing and temperatures near 0 °C inhibit growth and activity of aminogenic organisms and therefore constitute the most effective way to prevent biogenic amine accumulation in fresh products, and thus also in raw materials (Ruiz-Capillas and Jimenez Colmenero, 2010).

## 6.2. Fermentation

During fermentation, besides the contribution of contaminating bacteria, the microbiota responsible for fermentation can also show aminogenic activity. Moreover, the proteolytic activities, yeast lysis and acidification usually accompanying fermentation processes increase the availability of precursor free amino acids and favour decarboxylation reactions (ten Brink et al., 1990). Although biogenic amine contents in fermented products are highly variable, from not detected to more than 1000 mg/kg, the available data indicate that it is possible to produce fermented food without or with very low levels of biogenic amines. Therefore, the implementation of specific technological measures should mainly aim to control aminogenic microorganisms participating in the fermentation and ripening process.

The so-called "low histamine technology" (Bodmer et al., 1999) has been described based on both the preventive approach (through GHP, GMP and HACCP) as well as the implementation of specific technological measures. This technology has been successfully implemented for the manufacturing of traditional alcoholic beverages in certain countries (Bodmer et al., 1999). The challenge is to broaden this integrated approach to other biogenic amines to all types of food, whilst keeping the typical characteristics of the products (i.e. organoleptic, nutritional, etc.) expected by the consumers.

To control the fermentation, environmental conditions favouring a proper selection of desired fermentative (non-aminogenic) microorganisms and inhibition of undesired (aminogenic) microbiota should be managed. To this aim, principles of microbial ecology of food fermentation must be applied on the basis of product formulation (salt, sugar, preservatives, spices) and technological processing parameters (temperature, relative humidity, time, diameter/size, etc). The complexity of the fermentation enables the occurrence of a variety of additives, synergic or even antagonistic effects among ecological factors. Moreover, a given ecological factor can simultaneously show multiple targets or mechanisms of action. This is the case for the typical acidification accompanying nearly all, or most of, the fermentation processes. While a rapid and sharp decrease in pH is recognised as a key factor to reduce the growth of contaminating microbiota, it can also stimulate decarboxylation reactions in surviving microbiota as a response against unfavourable acidic environments (Vidal-Carou et al., 2007). Other technological variables such as the modification of the type or concentration of fermentable sugar, the addition of non-therapeutic antimicrobials (sulphite, etc.), or the use of relatively low processing temperatures have been presented as appropriate measures to prevent the accumulation of biogenic amines, not only during manufacture but also during storage of fermented products (Latorre-Moratalla et al., 2010a; Pinho et al., 2001, Suzzi and Gardini, 2003). Unfortunately, a common rule cannot be defined for each type of product because each food type needs specific formulation and processing parameters, which have to be assessed on a case-by-case basis.

Nowadays, the use of selected starter cultures is recognised as the most reliable approach to control the fermentation process, for both the large-scale and traditional small productions of fermented foods and beverages. However, microorganisms must be appropriately selected for each type of product and variety (i.e. substrate) taking into account their technological competence (competitiveness, influence on organoleptic characteristics, etc.) and safety requirements, including the inability to produce biogenic amines which is not a usual criteria for starter culture selection (Buckenhüskes, 1993, Holzapfel, 2002). Starter cultures should be ideally free of the potential to form biogenic amines, even if some bacterial starter cultures commercially used for food fermentation have been proved to produce biogenic amines (ten Brink et al., 1990).

For example, *Oenococcus oeni* the LAB that occurs naturally in wine, is the dominant bacterial species found during the malolactic fermentation (MLF), and it possesses the finest oenological malolactic characteristics. However, the main histamine producers in wine have been determined to belong to the species *O. oeni*. Therefore, prior to inoculation, MLF starters should be checked for their ability to produce histamine. According to the literature, *L. sakei* and *S. xylosus* can be suitable



starter cultures for fermented sausages since they are adapted to meat fermentation conditions and usually lack of aminogenic potential (Bover-Cid et al., 2000b,c; Latorre-Moratalla et al., 2009; Latorre-Moratalla et al., 2010b). However, given the heterogeneous distribution of amino acid decarboxylases among strains of a given species, it is necessary to proceed on a case-by-case basis and test the potential decarboxylase activity of any strain intended to be used as a starter culture. Moreover, the performance of the selected strain/s must be assessed in the real product under the actual processing conditions.

Starter cultures interact between different background microbial populations and thus they can influence the biogenic amine accumulation by the competitive suppression of amine-producing bacteria, limiting their growth and/or minimising their activity, resulting in the dominant microbial group without producing biogenic amines. Starter cultures could also modify the factors influencing biogenic amine formation (availability of precursor amino acids, acidification, etc.). Starter bacteria could even bear amine-degrading enzymes (MAO/DAO) contributing to biogenic amine catabolism *in situ* if they find proper conditions.

The protective performance of starter culture to prevent BA accumulation will strongly be conditioned by several features, including:

- Adaptation of strains to the particular fermentation ecology which can be better if strains are isolated from the same product or type of product. Standardized commercial preparations can be less effective than indigenous starters, although the influence is strain dependent (Latorre-Moratalla et al., 2010b). As an example: in fermented sausages, *L. sakei* and *L. curvatus* are well adapted to the meat fermentation environment, which make them good candidates for starter cultures since they are highly competitive to outgrow spontaneous fermenting flora and can efficiently inhibit Gram-negative contaminating bacteria (Hugas and Monfort, 1997). Indeed, the literature confirms that starter cultures including decarboxylase-negative strains of *L. sakei* are the most protective as they reduce the overall amine accumulation by up to 95 % in comparison with 30%-40% achieved with other commercial starters consisting of *L. plantarum* and *Pediococcus* spp. Mixed starter cultures, not only of lactic acid bacteria, but with other species involved in meat fermentation (e.g. staphylococci) will contribute to control a wider variety of aminogenic microorganisms (Vidal-Carou et al., 2007).
- The hygienic quality (initial microbial loads) of raw materials and ingredients, as discussed earlier. Optimal hygienic standards should be assured in order to facilitate the dominance of starter bacteria from the early stages of the fermentation (Bover-Cid et al., 2001b, Maijala et al., 1995a, Novella et al., 2004a,b).
- Optimization of the technological conditions to favour a proper implantation and development of the starter. The extent of aminogenesis reduction also depends on the formulation, including the type and amount of fermentable carbohydrate(s) (González-Fernández et al., 2003), the use of additives, including essential oils (Bozkurt and Erkmén, 2002, Latorre-Moratalla et al., 2010b), as well as the temperature and relative humidity of the ripening conditions (Bover-Cid et al., 2001a, Maijala et al., 1995b).

Therefore, it is of crucial importance to know the type of product and its productive process to find optimal intervention strategies. Since aminogenesis in fermented food and beverages is the result of complex phenomena affected by multiple factors, specific product/processor measures should be designed assessing and considering the particularities of the product, production process and processing environment characteristics.

## 7. Analytical methods

Detection of biogenic amines are important as part of investigating incidents of potential food-poisoning, for verification of the food production processes (including HACCP) and as a measure of quality (freshness) of both raw materials and finished products. Analytical methods for determination of BA in foods are reviewed in Rogers and Staruszkiewics (1997), Vale and Glória (1997), Önal (2007) and Sarkadi (2009).

### 7.1. Extraction techniques

Prior to detection, most analytical techniques require some form of extraction, although this has been reported as either matrix dependent or not necessary for some matrices (Önal 2007). Reported extraction procedures consist of the use of acids (trichloroacetic acid, hydrochloric, perchloric, thiodipropionic, or methansulfonic acids), solvents (petroleum ether, chloroform, or methanol) and filtration. The complexity of the varied food matrices is a critical consideration in obtaining adequate recoveries of all biogenic amines.

### 7.2. Biological methods

Since the consumer is unlikely to intentionally consume spoiled food, it has to be noted that seafood associated with histamine poisoning often appears organoleptically 'normal', even when containing high histamine concentrations, hence unacceptable and toxic levels of histamine are undetectable prior to consumption. This observation is in agreement with laboratory studies in that histamine concentrations above 500 mg/kg in seafood were organoleptically acceptable (Fletcher, 1995; Hwang et al., 1995; Lopez-Sabater et al., 1996).

The original analytical methods for biogenic amines in food relied detecting *in vivo* biological activity (AOAC, 1995a). For example, elevated levels of histamine in raw and canned sardines and mackerel using contraction of guinea pig ileum were reported (Geiger 1944; Geiger et al. 1944). However, these methods have now been superseded by *in vitro* methods largely based on chromatography, fluorometry or immunassays.

#### 7.2.1. Rapid and semi-quantitative methods

##### 7.2.1.1. Immunoassays

Immunoassays for histamine are commercially available from a variety of manufacturers (Appendix A). These assays are predominantly validated for application to seafood, applicable with minimal extraction, claimed to have varying levels of sensitivity (usually with a detection limit of around 0.5 mg/kg), are qualitative or quantitative, and usually relatively rapid. There are no commercially available assays for determining other BA in foods at the time of preparation of this document.

Immunoassays have the advantage of providing semi-quantitative data and can simultaneously analyse multiple samples within 20 minutes to 2 hours. This format provides a suitable alternative for screening for histamine (but not other biogenic amines) without the need to invest in additional equipment necessary for the fully quantitative assays.

##### 7.2.1.2. Flow injection analysis (FIA)

A flow injection analysis system for the determination of histamine using fluorimetric detection was developed to provide rapid screening of fish samples (Hungerford et al., 1990). The analytical technique is based on microfluidic manipulation of samples and reagents whereby samples are

injected into a carrier/reagent solution which transports the sample into a detector. During this transport the desired biochemical reactions takes place. For quantification of the target analyte a calibration curve for a given detector response (absorbance, fluorescence, sensor, etc.) is used. The technique does not require pre-treatment of the extract and care has to be taken to select the appropriate reagent concentrations and ensure that the carrier/reagent flow rate to maintain specificity for the histamine derivative (Hungerford et al., 1990). More recently, commercial FIA systems have been tested for screening of fishery products the method performance is satisfactory, with detection and quantitation limit from 0.8 mg/kg, and linearity to approximately 340 mg/kg (Hungerford et al., 2001).

#### 7.2.1.3. Colorimetric method

A colorimetric assay for histamine has recently been described based on the formation of a red complex between histamine and copper ions (Patange et al., 2005). The assay provides a detection limit of around 5 mg/kg and quantified either visually or by measuring the absorbance with a spectrophotometer at 496 nm. The assay is relative rapid (45 minutes), is inexpensive and does not require specialised laboratory equipment.

### 7.2.2. Quantitative methods

#### 7.2.2.1. Fluorometric methods

Fluorometric assay, as the AOAC Official Method (AOAC, 1995b) was widely used for determination of histamine in foods (usually fishery products) before the chromatographic procedures were described, and remains in the Codex Alimentarius list of methods of analysis (CODEX STAN 234-1999, amended in 2007) and is the reference method for the US Food and Drug Administration (Codex Alimentarius Commission, 1999; FDA, 2001). This method remains commonly in use in the US, Australia (Anonymous, 2001a) and South Africa (Anonymous, 2001b) as well as in some European laboratories. The method comprises a methanol extraction from fish which are subjected to an organic extraction. Histamine is derivatised with *o*-phthalaldehyde (sometimes after removal of interferences with an ion exchange column), to produce a fluorometric product which can be quantitatively determined on a spectrofluorometer (Taylor et al., 1978). This method is not suitable for the determination of biogenic amines other than histamine, and there is very limited validation data on this use of this method for matrices other than fish. The fluorometric assay has a detection limit around 0.5 mg/kg, provides linear sensitivities over wide ranges and has been subject to successful inter-laboratory trails. Although widely used in some parts of the world, the fluorometric assays require specialised equipment and should, where possible, be substituted for HPLC based methods which can quantify all biogenic amines.

#### 7.2.2.2. Chromatographic methods

Various chromatographic methods have been reported for determination of biogenic amines in foods including thin layer, capillary electrophoretic, gas, and high pressure liquid chromatography (HPLC). The HPLC is probably the most common technique used for biogenic amine determination in food. In Europe, the reference method specified in the European Commission Regulation (EC) No 2073/2005 (microbiological criteria for foodstuffs) for determination of histamine in fresh and treated fishery products is high pressure liquid chromatography (HPLC) after dansyl-derivatisation (Malle et al., 1996; Duflos et al., 1999). Various reagents for derivatization are also used (either pre or post column are used) including dansyl and dansyl chloride, benzoyl chloride, fluoresceine, 9 fluorenylmethyl chloromate, *o*-phthalaldehyde (OPA), naphthalene-2,3-dicarboxaldehyde, N-acetylcysteine, 2-mercaptoethanol. Dansyl chloride is probably the most widely used reagent for pre-column

derivatisation, while OPA is mostly used for post-column derivatisation. For detection, fluorescence, UV and electrochemical detection are most usually used.

HPLC based assay has a detection limit around 0.1 mg/kg, provides linear sensitivities over wide ranges and has been subject to successful inter-laboratory trials. The method requires specialised equipment and skills. Since HPLC method is cited as the reference method for histamine in Regulation (EC) No 2073/2005 (microbiological criteria for foodstuffs) and can quantify all biogenic amines, this should be the analytical method of choice for Official Control.

### 7.2.3. Detection of amino acid decarboxylase-positive microorganisms

Methods for the detection of biogenic amine producing microorganisms were initially based on the *in vitro* use of differential growth media containing specific substrates and a pH indicator to detect biogenic amine accumulation after the medium changes colour. Other alternative *in vitro* detection methods have also been described including CO<sub>2</sub> measurement, enzymatic or chemical analysis of biogenic amines (Marcobal et al., 2006c). The methods based on differential media have been reported as unreliable because of the generation of false negative and false positive results, as well as requiring the isolation and subsequent growth of the BA-producing microorganism, being time-consuming to perform. The chemical analysis of biogenic amine production in a decarboxylase broth constitutes a proper procedure to characterise and quantify the phenotypic expression of potentially aminogenic microorganisms.

A relationship between the presence of the gene encoding the decarboxylase and the capacity to synthesise BA has been shown by several authors. The characterization of the genes encoding decarboxylating enzymes led the development of sensitive and specific methods based on PCR, hence the detection of these genes by PCR enable to identify organisms with a potential to produce BA more rapidly than other *in vitro* tests. However, PCR based tests have the disadvantages that they only indicate the potential for BA production (and not production of biologically active enzyme) as well still requiring *in vitro* culture, equipment and trained analysts for DNA extraction, amplification and PCR product detection. PCR has also been used to quantify and detect decarboxylase genes directly in food. However, the amplified PCR fragments may suggest the presence of BA producing microorganisms but it does not allow identifying the production source (microorganism). Therefore a multiphasic approach (isolation of microorganisms and detection of genes involved in BA production) it is always suggested.

Decarboxylase genes suitable for the design of specific primers for detecting potential BA producing strains by PCR have been undertaken (Landete et al., 2007a). PCR based protocols have been described, both using 'block-based' as well as real time PCR assays. The latter having the advantage of being able to more readily quantify the amount of target in the original sample. Monoplex as well as multiplexed assays for different genes have also been described (Landete et al., 2007a). Because of the high sequence diversity of genes encoding decarboxylases, a diverse range of PCR assays is required to amplify and detect fragments from all major enzyme groups (see section 2.2.1). However assays for the seven broad classes of enzymes have been described which include: i. pyridoxal phosphate-dependent histidine decarboxylase (Gram-negative bacteria); ii. pyruvoyl-dependent histidine decarboxylase (Gram-positive bacteria); iii. pyridoxal-dependent tyrosine decarboxylase (Gram-positive bacteria); iv and v. ornithine decarboxylases, one for Gram-positive and Gram negative bacteria and a second, and rarer enzyme confined to Gram-negative bacteria; vi and vii. two lysine decarboxylases (one confined to Gram-positives the other to Gram-negative bacteria) (de las Rivas et al., 2006; Marcobal et al., 2005; Landete et al., 2007b,c). For example, a multiplex PCR method for the simultaneous detection of LAB with the potential to produce histamine-, tyramine and putrescine- has been described (Marcobal et al., 2005). Furthermore, a quantitative real-time PCR for histamine-producing LAB (Fernández et al., 2006; Lucas et al., 2008) and for tyramine-producing bacteria (Torriani et al., 2008) has also been successfully applied to different stages of cheese

manufacture including the final product (Ladero et al., 2008; Ladero et al., 2010b; Gardini et al., 2008). Molecular methods have also been designed to study histidine- and tyrosine- decarboxylase gene expression under conditions relevant for food fermentation (Rossi et al., 2011), including cheese making and sausage fermentation (Gardini et al., 2008), respectively.

These PCR methods may be used for the characterization and selection of starter cultures but have also been used for the early detection of BA producers within a food production process. In the latter situation, because of the high sequence diversity of genes encoding decarboxylases, several false positive arise from PCR based reactions. Therefore, sequence analysis of PCR fragments potentially related to BA coding genes, is recommended.

### 7.3. Monitoring and surveillance

Monitoring and surveillance of food poisoning is undertaken by Competent Bodies in each MS although the exact mechanisms for this vary considerably between MSs. The majority of incidents of food poisoning due to BA are reported by passive surveillance, and comprise reports of sporadic or outbreaks of compatible human disease associated with elevated levels of histamine in fishery products. Reporting of human disease takes place from clinical laboratories and /or public health organisations and surveillance data may be made available in a collated form. These surveillance data is complemented by analysis of food which is carried out by Official Control Laboratories (as defined in European Regulation (EC) No 882/2004). The Official Control Laboratories are also involved with statutory food control. The presence of histamine in fishery products is covered under European Commission Regulation (EC) No 2073/2005 (microbiological criteria for foodstuffs, see previous section) and adverse incidents are reported by Competent Bodies through RASFF reporting, as outlined in Section 4.1.1. The public health organisations involved in human disease surveillance may differ from the Competent Bodies in some MSs. Under Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents the Competent Body in each MS is required to report food-borne outbreaks to the European Centre for Disease Prevention and Control (ECDC), who collates data on cases of zoonoses, antimicrobial resistance and foodborne outbreaks. The EFSA is then responsible for preparing the Community Summary Report (EFSA, 2010a) on the basis of the zoonoses data. This includes foodborne outbreaks due to histamine poisoning, which also includes data on locations, settings and contributory factors. No reports of intoxications due to BA other than histamine have been reported through the Zoonoses Monitoring process.

#### 7.3.1. EU legislative requirements and guidances

Specific legislation which includes microbial criteria for BA in foods only covers histamine in fishery products (which is not within the scope of this document): criteria for other BA or other food products do not occur in any national legislation. In the EU, European Commission Regulation (EC) No 2073/2005 (as well as its amendments such as Regulation (EC) No 1441/2007 and Regulation (EU) No 365/2010) sets food safety criteria for histamine in two different fishery products. The first is for fish species (particularly within the families *Scombridae*, *Clupeidae*, *Engraulidae*, *Coryphenidae*, *Pomatomidae*, and *Scombreresosidae*) placed on the market during their shelf life and, with a sampling plan comprising nine units, two of the units may be between 100 and 200 mg/kg of histamine, and none above the limit of 200 mg/kg. This regulation also applies to fishery products (particularly for the families given above) which have undergone enzyme maturation treatment in brine and placed on the market during their shelf life and, with a sampling plan of nine units, two of the units may be between 200 and 400 mg/kg of histamine, and none above the limit of 400 mg/kg. For both these products, the analytical method specified in Commission Regulation (EC) No 2073/2005, is by HPLC (Malle et al., 1996; Duflos et al., 1999).

The USFDA (Anonymous, 2001c) considers that although “chemical testing is an effective means of detecting the presence of histamine in fish flesh, the validity of such testing is dependent upon the



design of the sampling plan. The amount of sampling required to accommodate such variability is necessarily quite large. For this reason, chemical testing alone will not normally provide adequate assurance that the hazard has been controlled. Because histamine is generally not uniformly distributed in a decomposed fish, a guidance level of 50 ppm (50 mg/kg) has been set. If 50 ppm is found in one section, there is the possibility that other sections may exceed 500 ppm (500 mg/kg).” The analytical method is the official fluorimetric method (AOAC, 1995b). The Australian and New Zealand Food Standards Code (Volume 2, Standard 2.2.3 (Page 22301, September 2002) states that ‘the level of histamine in fish or fish products must not exceed 200 mg/kg’ (Bremer et al., 2003).

The Codex Alimentarius Standards for fish provide histamine levels as indicators for (1) decomposition and (2) hygiene and handling. For decomposition, the relevant standards state”: ‘The products shall not contain more than 10 mg/100 g (100 mg/kg) of histamine based on the average of the sample unit tested. This applies only to species of *Clupeidae*, *Scrombridae*, *Scromberesocidae*, *Pomatomidae* and *Coryphaenidae* families.’ For hygiene and handling, the relevant standards state: ‘No sample unit shall contain histamine that exceeds 200 mg/kg. This applies only to species of the families *Scrombridae*, *Clupeidae*, *Coryphaenidae*, *Scrombreresocidae* and *Pomatomidae*.’ These Codex Standards cover: quick frozen fish fillets, quick frozen blocks of fish fillet, minced fish flesh and mixtures of fillets and minced fish flesh, eviscerated and uneviscerated quick frozen finfish, quick frozen fish sticks (fish fingers), fish portions and fish fillets - breaded or battered, canned sardines and sardine type products, and canned tuna and bonito (Bremer et al., 2003).



## CONCLUSIONS

*ToR1: Carry out a review of the available published scientific information on biogenic amines in foods with regards to production, processing, transport, storage/retail (i.e. during the shelf-life of the products) until consumption; including on consumer exposure and potential health implications.*

- The present knowledge and data on toxicity of biogenic amines (BA) individually and in combination(s) are limited: nevertheless, histamine and tyramine are considered as most toxic and particularly relevant for food safety.
- The main pre-requisites for the presence of BA in foods include: availability of free amino acids, the presence of microorganisms producing BA enzymes (mainly from raw materials and/or added starter cultures), and conditions allowing their growth (particularly temperature, pH), as well as conditions affecting the enzyme production and activity (particularly low pH).
- Fermentation of foods provides the conditions indicated above allowing intensive microbial activity and therefore has the potential for BA formation.
- Storage and distribution conditions (in particular temperature) for fermented foods are variable in practice and may be relevant for BA accumulation.

*ToR2: Carry out a risk profiling of relevant fermented foods regarding biogenic amine formation from production to consumption (within their 'use-by' date). Include data from additional sources where applicable. The profiling should also result in identification of data needs to support a quantitative risk assessment.*

- Among groups of foods and within each group indicated below there is variability in amounts of BA found. Data are inadequate for differentiating individual products within each group.
- Based on the mean content of the most toxic BA (histamine and tyramine), the food safety relevance of the considered food categories can be ranked in following decreasing order:

for **histamine**: 'dried anchovies', 'fish sauce', 'fermented vegetables', 'cheese', 'other fish and fish products' (as indicated on page 31 in section 4.1.2.) and 'fermented sausages';

for **tyramine**: 'fermented sausages', 'fish sauce', 'cheese', 'fermented fish' and 'fermented vegetables'.

- Based on the consumer exposure to the most toxic BA, the food safety relevance of the considered food categories can be ranked in following decreasing order:

for **histamine**: 'other fish and fish products', 'fermented sausages', 'cheese', 'fish sauces' and 'fermented vegetables';

for **tyramine**: 'beer', 'cheese', 'fermented sausages', 'fermented fish meat' and 'preserved meat'.

- For quantitative risk assessment, further information and data on BA (individually and in combination(s)) including toxicity, concentration and consumption of fermented foods are required.

*ToR3: Identify and rank possible risk mitigation options and their anticipated impact to prevent or limit biogenic amines formation in fermented foods.*

- BA accumulation in fermented foods is a complex process affected by multiple factors and their interactions, the combinations of which are numerous, variable and product-specific. Hence, risk mitigation options, which are based on controlling those factors/interactions, could not be considered and ranked individually. Rather, they were considered at general principles level:
  - Minimizing the occurrence of BA-producing microorganisms can be achieved through ensuring the good hygienic status of the raw material and, where possible, additional microbial controls.
  - Microorganisms intended to be used as starter cultures in any fermented food should be confirmed as not producing BA and able to outgrow autochthonous microbiota under conditions of production and storage.
  - All aspects of fermented food processing (including ingredients, fermentation and ripening regimes), distribution and storage should be adjusted and balanced in each particular product to avoid/minimize potential enhancing effects on BA formation and to enable dominance of starter culture(s) where used.

*ToR4: Characterise concentration levels of biogenic amine in relevant fermented foods that are not associated with adverse health effects to defined consumer groups including susceptible consumers.*

- Estimating safe levels of the total amounts of BA ingested is the key issue to understand health effects to consumers. Consumption data and the exposure assessment were used by the Panel to define the concentrations in food that would be allowable, however these will vary between individuals, regions and countries. Therefore, for the purpose of this document, the focus was on total amounts of BA ingested in relation to estimated threshold levels for BA.

## Histamine

Currently, available information needed for establishing NOAEL and ARfD in humans is based only on limited number of healthy and sensitive individuals.

- Based on limited published information, no adverse health effects have been observed in healthy volunteers exposed to a level of 25 to 50 mg of histamine per person per meal. This level may be occasionally exceeded by consumption of one or more food items containing high amounts of histamine during the same meal.
- In patients with histamine intolerance, even small amounts of histamine in ingested food may cause adverse health effects, so only levels below detectable limits can be considered as safe.

## Tyramine

There is currently insufficient information related to establishing a NOAEL in humans.

- Based on limited published information, no adverse health effects have been observed in healthy individuals not taking monoamino oxidase inhibitor (MAOI) drugs exposed to a level of 600 mg of tyramine per person per meal. This level would not be exceeded even by a combined high intake of the five main food sources of tyramine during the same meal.
- In individuals taking third generation MAOI drugs, no adverse health effects have been observed after exposure to a level of 50 mg of tyramine per person per meal. High consumption of some

fermented foods (beer, cheese, fermented sausages and fermented fish meat) can lead to tyramine exposure exceeding this level.

- For individuals taking classical MAOI drugs, no adverse health effects have been observed after exposure to a level of 6 mg of tyramine per person per meal. This would be easily exceeded by the consumption of fermented food.

#### Putrescine and cadaverine

- For putrescine and cadaverine, presently available information is insufficient to identify concentrations that directly cause acute adverse health effects and/or potentiate the toxic effects of histamine and other biogenic amines.

*ToR5: Give advice regarding the analytical method to measure biogenic amine concentrations in fermented foods.*

- Presently, high-performance liquid chromatography (HPLC)-based methods are the only methods which reliably and with high sensitivity can simultaneously quantify concentrations of all BA in fermented food, therefore, are most suitable for analysis of fermented foods.

*ToR6: Recommend the monitoring methods in fermented foods that are most relevant from the public health point of view. These recommendations may refer to, among other aspects, the fermented food categories, the stages during food production until consumption within the shelf-life of a product to be sampled, as well as the type of sample to be collected.*

- Currently, there is insufficient information in order to recommend detailed monitoring schemes and methods.
- Monitoring of BA concentrations in fermented food during the production process could be used as one of the parameters for the process hygiene assessment.
- Monitoring of raw materials and products at multiple points along the food chain is necessary to evaluate the relevance of various factors contributing to BA formation and accumulation in fermented foods.

## RECOMMENDATIONS

- Further research is needed on:
  - the toxicity and associated concentrations of histamine and tyramine in different foods, as well as related potentiating effects of putrescine and cadaverine, in particular concerning food involved in outbreaks and sporadic cases;
  - the consumption data of fermented foods, especially cheese;
  - the production process-based control measures for BA in fermented food including monitoring and verification aspects and the development of challenge tests;
  - the evaluation of the need for and, if/where necessary, development of process hygiene criteria for histamine and tyramine in fermented foods, as well as food safety criteria for histamine in fermented foods other than fish.
- Validation of methods for BA analysis is recommended for all relevant food types including standardisation and harmonisation of procedures, external quality assessment and availability of certified reference materials.

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## APPENDICES

### A. APPENDIX EXAMPLES OF COMMERCIALY AVAILABLE IMMUNOASSAYS FOR HISTAMINE

Test*	Analytical Technique	Approx. Total Test Time	Supplier*
ALERT® for Histamine [Sensitivity: 2.5 ppm]	ELISA	35 min	Neogen Corporation www.neogen.com
EIA for Histamine in Fish Extract, K1-HTM [Sensitivity: 2.5 ppm, quantitative 1-50 ppm]	Enzyme immunoassay	90 min	Immuno-Diagnostic Reagents www.idr-usa.com
EIA for Histamine Fishmeal and Bonemeal, K2-HTM [Sensitivity: 5 ppm, qualitative]	Enzyme immunoassay	35 min	Immuno-Diagnostic Reagents www.idr-usa.com
EIA for Histamine in Raw and Canned fish, K3-HTM [Sensitivity: 5 ppm, qualitative]	Enzyme immunoassay	35 min	Immuno-Diagnostic Reagents www.idr-usa.com
HISQUICK™ Histamine (BA-20-3000 - 48 Columns) [Sensitivity: 20 ppm, quantitative]	Color test	12 min.	Rocky Mountain Diagnostics, Inc. www.rmdiagnosics.com
Histamine EIA Food (BA-10-3100 - 96 Wells) [Sensitivity: 0.5 ppb, quantitative]	Enzyme immunoassay	2 h	Rocky Mountain Diagnostics, Inc. www.rmdiagnosics.com
Histamarine Test Kit1 (AOAC Approved) [Sensitivity: 0.5 ppm, quantitative from 1 to 500 ppm]	Enzyme immunoassay	1 h	Immunotech (Beckman Coulter) www.immunotech.cz
HistaMeter [Sensitivity: 0-50 ppm, qualitative]	Enzyme immunoassay	1 h	Biomedix E-mail: cb4biomedx@aol.com
HistaQuant [Sensitivity: 0-500 ppm, quantitative]	Enzyme immunoassay	1-1/2 h	Biomedix E-mail: cb4biomedx@aol.com
HistaSure Dipstick Assay [5ppm Pass/Fail]	Fluorescence Labeled Optical-Read Immuno Dipstick Assay (F.L.O.R.I.D.A.)	5 min	Rocky Mountain Diagnostics www.rmdiagnosics.com
RIDASCREEN® Histamin R1602 [Sensitivity: 2.5 ppm; quantitative]	ELISA	2-5/6h	R-Biopharm, Inc. www.r-biopharm.com/main.php?
RidaQuick Histamin (R1603-96 Wells) [Sensitivity 20 ppm; quantitative]	ELISA	12 min	R-Biopharm, Inc. www.r-biopharm.com/main.php?
Transia Tube Histamine	ELISA	1 h	Diffchamb AB www.bestlab.com.au/diffchamb.htm
Veratox® for Histamine [Sensitivity: < 2.5 ppm, quantitative from 0 to 50 ppm]	ELISA	35 min	Neogen Corporation www.neogen.com

\*Information based on <http://seafood.ucdavis.edu/haccp/compendium/chapt27.htm#Commercial%20Test%20Products>

## B. APPENDIX CONSUMPTION DATA EXTRACTED FROM THE EFSA COMPREHENSIVE FOOD CONSUMPTION DATABASE

Further information on the surveys listed here, identified by their respective acronyms, can be found in the ‘Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment’ (European Food Safety Authority; Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. EFSA Journal 2011;9(3):2097. [34 pp.] doi:10.2903/j.efsa.2011.2097. Available online: [www.efsa.europa.eu/efsajournal.htm](http://www.efsa.europa.eu/efsajournal.htm))

Food category	Sub-category	Austria				Belgium				Bulgaria			
		ASNS				Diet_National_2004				NSFIN			
		Cons. days	% total days	Amount (g/day)		Cons. days	% total days	Amount (g/day)		Cons. days	% total days	Amount (g/day)	
				mean	P95			mean	P95			mean	P95
Alcoholic beverages	Beer	274	12.91%	648.16	1500	485	18.32%	689.39	2000	111	16.06%	572.97	1500
	Fortified and liqueur wines	.	.	.	.	89	3.36%	105.02	250	.	.	.	.
	Wine, red	267	12.58%	176.29	500	477	18.01%	280.36	633.2	20	2.89%	52.3	270
	Wine, white	30	1.41%	275.17	630	176	6.65%	235.18	500	19	2.75%	231.38	1500
	Wine, white, sparkling	23	1.08%	135.87	300	31	1.17%	179.44	400	.	.	.	.
Fish and fish products	Fermented fish products	210	9.89%	135.88	293	617	23.30%	99.46	246.8	71	10.27%	193.56	413.6
	Other fish products	25	1.18%	8.96	8	96	3.63%	54.47	180	.	.	.	.
Meat and meat products	Fermented sausages	653	30.76%	57.36	145	293	11.06%	46.42	142.5	37	5.35%	60.13	150
	Other ripened meat products	451	21.24%	51.74	150	956	36.10%	52.83	135	85	12.30%	44.31	100
	Other meat products	825	38.86%	90.65	240	487	18.39%	90.58	227.5	123	17.80%	109.85	300
Milk and milk products	Cheese	1335	62.88%	63.06	174	1641	61.97%	69.06	200	381	55.14%	57.06	150
	Yoghurt	620	29.20%	210.08	500	593	22.39%	167.66	347.6	331	47.90%	173.33	498.5
	Other dairy products	1528	71.97%	123.47	489	1564	59.06%	158.26	515	273	39.51%	115.88	327.5
Savoury sauces	Fish sauce	36	1.70%	9.33	21	30	1.13%	19.57	50	.	.	.	.
	Other savoury sauces	6	0.28%	81.33	148	427	16.13%	72.52	192	.	.	.	.
Vegetables and vegetable products	Fermented vegetables	516	24.31%	32.62	121	252	9.52%	38.98	140	202	29.23%	11.45	20
	Other vegetables	1003	47.24%	54.4	108	622	23.49%	49.98	160	226	32.71%	103.71	301.7

Survey		Czech Republic				Denmark				Estonia			
		SISP04				Danish Dietary Survey				NDS_1997			
		Cons. days	% total days	Amount (g/day)		Cons. days	% total days	Amount (g/day)		Cons. days	% total days	Amount (g/day)	
Food category	Sub-category			mean	P95			mean	P95			mean	P95
Alcoholic beverages	Beer	1170	35.11%	1108.46	3000	4813	24.40%	791.05	2640	218	11.68%	1027.09	3000
	Fortified and liqueur wines	.	.	.	.	306	1.55%	74.99	150	7	0.38%	164.29	300
	Wine, red	187	5.61%	328.29	700	4553	23.09%	353.58	700	59	3.16%	323.31	1000
	Wine, white	212	6.36%	307.26	800	1414	7.17%	273.55	560	2	0.11%	210	300
	Wine, white, sparkling	.	.	.	.	.	.	.	.	25	1.34%	246	700
Fish and fish products	Fermented fish products	362	10.86%	145.5	300	7886	39.99%	45.56	145	297	15.92%	130.05	329.4
	Other fish products	25	0.75%	121.58	300	.	.	.	.	10	0.54%	65	150
Meat and meat products	Fermented sausages	518	15.55%	73.37	198	5585	28.32%	14.13	43.4	.	.	.	.
	Other ripened meat products	976	29.29%	59.18	200	10713	54.32%	13.68	39	334	17.90%	86.84	283.6
	Other meat products	1431	42.95%	119.54	294	6479	32.85%	54.53	210.58	903	48.39%	98.26	280
Milk and milk products	Cheese	1587	47.63%	65.14	150	15655	79.38%	39.33	108	648	34.73%	75.79	246.95
	Yoghurt	694	20.83%	201.98	500	6304	31.96%	149.1	380	366	19.61%	291.33	750
	Other dairy products	2450	73.53%	134.11	500	18302	92.80%	320.13	954	1388	74.38%	277.27	772.7
Savoury sauces	Fish sauce	52	1.56%	11.25	30.77	117	0.59%	3.38	19.2	112	6.00%	7.89	16
	Other savoury sauces	.	.	.	.	.	.	.	.	.	.	.	.
Vegetables and vegetable products	Fermented vegetables	1187	35.62%	65.44	200	1774	9.00%	3.5	8.86	128	6.86%	49.56	210
	Other vegetables	168	5.04%	84.52	177.4	8096	41.05%	27.2	68	323	17.31%	18.44	43.2

Survey		Finland				France				Germany			
		FINDIET_2007				INCA2				National Nutrition Survey II			
		Cons. Days	% total days	Amount (g/day)		Cons. days	% total days	Amount (g/day)		Cons. days	% total days	Amount (g/day)	
Food category	Sub-category			mean	P95			mean	P95			mean	P95
Alcoholic beverages	Beer	413	13.11%	804.98	2500	1008	6.41%	415.81	1000	4633	22.23%	806.71	2000
	Fortified and liqueur wines	45	1.43%	25.1	110	384	2.44%	84.65	200	39	0.19%	125.86	350
	Wine, red	163	5.17%	241.15	720	3382	21.50%	264.51	600	2689	12.90%	282.11	700
	Wine, white	72	2.29%	193.63	480	777	4.94%	187.44	450	864	4.15%	287.69	625
	Wine, white, sparkling	.	.	.	.	461	2.93%	205.77	450	.	.	.	.
Fish and fish products	Fermented fish products	796	25.27%	102.34	245.27	4472	28.44%	95.58	224	2682	12.87%	115.93	285
	Other fish products	10	0.32%	20.4	45	517	3.29%	94.45	200	326	1.56%	136.65	285
Meat and meat products	Fermented sausages	269	8.54%	26.23	70	1298	8.25%	35.15	88	4329	20.77%	36.83	91
	Other ripened meat products	1228	38.98%	38.67	100	5385	34.24%	49.06	133	5960	28.60%	39.29	100
	Other meat products	858	27.24%	96.89	280	4025	25.59%	73.9	200	8878	42.60%	89.93	244.5
Milk and milk products	Cheese	2302	73.08%	50.54	134	10561	67.15%	70.98	200	13536	64.96%	57.83	172
	Yoghurt	1415	44.92%	252.88	600	5869	37.32%	172.38	375	5224	25.07%	188.95	500
	Other dairy products	2843	90.25%	333.4	923.24	8983	57.12%	156.07	450	13782	66.14%	155.37	525.3
Savoury sauces	Fish sauce	142	4.51%	7.36	20.4	106	0.67%	17.2	45	203	0.97%	16.17	45
	Other savoury sauces	.	.	.	.	1403	8.92%	37.88	90	3401	16.32%	65.07	153
Vegetables and vegetable products	Fermented vegetables	1098	34.86%	39.18	120	1802	11.46%	40.64	200	2220	10.65%	85.68	251.2
	Other vegetables	1203	38.19%	26.38	72	5579	35.47%	59.29	200	6307	30.27%	90.01	208.4

Food category	Survey	Hungary				Ireland				Italy			
		National_Repr_Surv				NSIFCS				INRAN_SCAI_2005_06			
		Cons. days	% total days	Amount (g/day)		Cons. days	% total days	Amount (g/day)		Cons. days	% total days	Amount (g/day)	
	Sub-category			mean	P95			mean	P95			mean	P95
Alcoholic beverages	Beer	268	8.32%	579.89	1000	1118	16.67%	1873.45	5044	702	10.12%	333.47	800
	Fortified and liqueur wines	.	.	.	.	161	2.40%	16.94	50	3	0.04%	120	120
	Wine, red	109	3.38%	225.51	500	413	6.16%	228.73	562	2141	30.85%	177.64	480
	Wine, white	162	5.03%	249.72	500	305	4.55%	241.3	625	1727	24.89%	64.36	250
	Wine, white, sparkling	11	0.34%	222.73	400	11	0.16%	165.91	450	.	.	.	.
Fish and fish products	Fermented fish products	150	4.66%	188.84	400	1127	16.81%	126.75	308.62	2247	32.38%	140.3	361
	Other fish products	.	.	.	.	2	0.03%	62.5	85	58	0.84%	121.27	243.84
Meat and meat products	Fermented sausages	538	16.70%	64.08	150	72	1.07%	24.4	87	617	8.89%	29.25	64
	Other ripened meat products	909	28.21%	57.7	200	3119	46.51%	84.94	249.18	2622	37.79%	49.69	106.25
	Other meat products	1265	39.26%	113.98	250	1292	19.27%	81.62	182.93	584	8.42%	100.85	216.94
Milk and milk products	Cheese	1268	39.35%	60.77	150	2444	36.44%	45.29	112	5299	76.37%	78.05	196.88
	Yoghurt	541	16.79%	198.83	400	788	11.75%	132.04	250	1002	14.44%	151.28	300
	Other dairy products	2700	83.80%	241.96	650	6400	95.44%	283.4	690.47	4360	62.83%	165.74	300
Savoury sauces	Fish sauce	1	0.03%	20	20	247	3.68%	11.64	35.05	17	0.24%	7.69	27
	Other savoury sauces	3	0.09%	10	10	1123	16.75%	58.46	120	.	.	.	.
Vegetables and vegetable products	Fermented vegetables	1289	40.01%	111.28	250	933	13.91%	13.04	40	55	0.79%	37.69	128
	Other vegetables	114	3.54%	92.85	200	1371	20.44%	21.82	60	3743	53.94%	84.56	250

Food category	Survey	Latvia				Netherlands				Poland			
		EFSA_TEST				DNFCS_2003				IZZ_FAO_2000			
		Cons. days	% total days	Amount (g/day)		Cons. days	% total days	Amount (g/day)		Cons. days	% total days	Amount (g/day)	
				mean	P95			mean	P95			mean	P95
Alcoholic beverages	Beer	243	9.15%	652.9	1200	247	16.47%	1196.86	4320	271	10.72%	615.31	1000
	Fortified and liqueur wines	1	0.04%	100	100	34	2.27%	29.65	236.3	.	.	.	.
	Wine, red	46	1.73%	221.3	500	75	5.00%	262.72	613.3	24	0.95%	130.21	200
	Wine, white	1	0.04%	50	50	46	3.07%	263.19	750	22	0.87%	168.18	500
	Wine, white, sparkling	13	0.49%	240.77	400	2	0.13%	149.4	253.8	10	0.40%	133	330
Fish and fish products	Fermented fish products	393	14.80%	110.41	250	126	8.40%	72.35	210	298	11.79%	159.49	404
	Other fish products	39	1.47%	145.9	360	26	1.73%	157.12	300	3	0.12%	38.33	50
Meat and meat products	Fermented sausages	299	11.26%	65.62	160	161	10.73%	35.41	90	43	1.70%	34.3	70
	Other ripened meat products	173	6.52%	83.15	200	595	39.67%	40.1	100.68	979	38.74%	66.3	175
	Other meat products	885	33.33%	98.75	240	496	33.07%	72.96	216	1447	57.26%	110.67	300
Milk and milk products	Cheese	1042	39.25%	72.07	200	956	63.73%	58.68	165	1154	45.67%	79.3	200
	Yoghurt	547	20.60%	242.46	500	560	37.33%	304.25	796.2	250	9.89%	189.13	450
	Other dairy products	1511	56.91%	108.31	380	1062	70.80%	317.48	873.07	1837	72.69%	152.7	500
Savoury sauces	Fish sauce	1	0.04%	50	50	111	7.40%	13.05	47.91	.	.	.	.
	Other savoury sauces	249	9.38%	62.55	110	444	29.60%	55.32	150	.	.	.	.
Vegetables and vegetable products	Fermented vegetables	55	2.07%	125.36	300	260	17.33%	39.49	127.79	1277	50.53%	80.93	233.55
	Other vegetables	40	1.51%	63.13	200	287	19.13%	80.98	263.69	107	4.23%	44.37	135.65



Food category	Sub-category	Slovakia				Slovenia				Spain							
		Survey				Survey				Survey							
		SK_MON_2008				CRP_2008				AESAN				AESAN_FIAB			
		Cons. days	% total days	Amount (g day)		Cons. days	% total days	Amount (g day)		Cons. days	% total days	Amount (g day)		Cons. days	% total days	Amount (g day)	
		mean	P95			mean	P95			mean	P95			mean	P95		
Alcoholic beverages	Beer	292	10.57%	724.16	2000	20	4.91%	552.5	1500	156	18.84%	464.42	1233	297	10.81%	350.78	750
	Fortified and liqueur wines	1	0.04%	100	100	1	0.25%	66	66	8	0.97%	83.21	200	21	0.76%	106.85	250
	Wine, red	71	2.57%	258.45	500	39	9.58%	195.5	500	109	13.16%	148.91	400	494	17.98%	154.65	500
	Wine, white	81	2.93%	258.89	500	19	4.67%	164.59	500	49	5.92%	55.71	240	325	11.83%	94.02	320
	Wine, white, sparkling	5	0.18%	170	250	.	.	.	.	.	.	.	.	.	.	.	.
Fish and fish products	Fermented fish products	128	4.63%	126.16	300	41	10.07%	117.84	240	450	54.35%	113.65	292.25	1570	57.13%	131.01	316.2
	Other fish products	63	2.28%	154.6	200	2	0.49%	35	50	31	3.74%	63.06	200	22	0.80%	70.91	200
Meat and meat products	Fermented sausages	522	18.89%	100.61	250	39	9.58%	59.49	100	185	22.34%	45.23	109.9	894	32.53%	36.71	98
	Other ripened meat products	515	18.64%	79.02	200	62	15.23%	66.16	170	402	48.55%	58.73	150	1480	53.86%	53.61	135.77
	Other meat products	573	20.74%	109.01	300	121	29.73%	98.19	220	114	13.77%	75.22	191.4	343	12.48%	67.73	170
Milk and milk products	Cheese	825	29.86%	61.64	150	138	33.91%	58.54	155.4	412	49.76%	54.31	147.5	1298	47.23%	47.43	123.66
	Yoghurt	667	24.14%	195.6	500	113	27.76%	221.08	500	362	43.72%	171.09	325	995	36.21%	153.91	250
	Other dairy products	1078	39.02%	179.32	530	213	52.33%	189.21	507.8	726	87.68%	280.9	600	2478	90.17%	336.68	750
Savoury sauces	Fish sauce	9	0.33%	10.89	30	1	0.25%	0.7	0.7	6	0.72%	14.69	25	3	0.11%	17.5	30
	Other savoury sauces	26	0.94%	143.08	280	28	6.88%	81.07	120	37	4.47%	35.59	100	44	1.60%	44.8	100
Vegetables and vegetable products	Fermented vegetables	550	19.91%	82.79	200	33	8.11%	142.8	274.6	16	1.93%	16.09	30	29	1.06%	20.52	75
	Other vegetables	105	3.80%	104.58	210	216	53.07%	99.56	181.2	372	44.93%	73.23	159.62	1257	45.74%	87.95	220.9

Food category	Survey Sub-category	Sweden				United Kingdom			
		Riksmaten 1997_98				NDNS			
		Cons. days	% total days	Amount (g/day)		Cons. days	% total days	Amount (g/day)	
				mean	P95			mean	P95
Alcoholic beverages	Beer	2367	27.96%	501.34	1200	2019	16.73%	1273.05	3444
	Fortified and liqueur wines	52	0.61%	135.87	400	231	1.91%	97.6	300
	Wine, red	1033	12.20%	322.89	600	1226	10.16%	274.22	750
	Wine, white	8	0.09%	306.25	950	873	7.23%	301.25	750
	Wine, white, sparkling	.	.	.	.	105	0.87%	292.17	740
Fish and fish products	Fermented fish products	2176	25.70%	87.33	210	2707	22.43%	113.67	255
	Other fish products	379	4.48%	117.77	150	227	1.88%	88.57	200
Meat and meat products	Fermented sausages	22	0.26%	31.41	75	377	3.12%	13.5	50
	Other ripened meat products	693	8.19%	83.01	150	4003	33.17%	56.25	155
	Other meat products	5008	59.15%	68.58	180	1704	14.12%	79.19	177
Milk and milk products	Cheese	5824	68.79%	42.46	100	4795	39.73%	51.24	127
	Yoghurt	2829	33.42%	273.13	500	1863	15.44%	138.82	258
	Other dairy products	5441	64.27%	371.98	950	10960	90.82%	245.09	601
Savoury sauces	Fish sauce	7	0.08%	15.71	35	383	3.17%	6.4	18
	Other savoury sauces	996	11.76%	98.92	200	2512	20.82%	76.07	190
Vegetables and vegetable products	Fermented vegetables	362	4.28%	37.63	96	2055	17.03%	24.9	114
	Other vegetables	312	3.69%	26.41	60	3279	27.17%	39.63	108

**GLOSSARY AND ABBREVIATIONS**

ACDC	amino acid decarboxylase
ARfD	acute reference dose
BA	biogenic amine
BAI	biogenic amine index
DAO	diamino oxidase
ECDC	European Centre of Disease Prevention and Control
EFSA	European Food Safety Authority
GHP	good hygiene practice
GMP	good manufacturing practice
HdcP	histidine/histamine antiporter
HDC	histidine decarboxylase
HNMT	histamine-N-methyltransferase
HPLC	high pressure liquid chromatography
LAB	lactic acid bacteria
LOD	limit of detection
LOQ	limit of quantification
MAO	monoamine oxidase
MAOI	monoamine oxidase inhibitor
MLF	malolactic fermentation
MS/MSs	Member State / Member States
NOAEL	no observed adverse effect level
OPA	<i>o</i> -phtalaldehyde
PLP	pyridoxal-5'-phosphate
PMF	proton motive force
P95	95-percentile
RIMA	reversible inhibitors of MAO-A
RASFF	Rapid Alert System for Food and Feed

RSD	relative standard deviation
TDC	tyrosine decarboxylase
ToR	Terms of reference
UB	upper bound: the value assigned to the limit of detection (LOD) to values reported as non-detected and the value of the limit of quantification (LOQ)