

ADVISORY COMMITTEE ON THE MICROBIOLOGICAL SAFETY OF FOOD**DISCUSSION PAPER****Histamine in cheese****Issue**

Poisoning by the biogenic amine histamine is a well-recognised phenomenon that arises from the consumption of food, particularly certain types of scombroid fish, which can have high levels of histamine present as a result of bacterial spoilage. Histamine can also be present as a consequence of fermentation in the production of foods such as certain cheeses or sausages. Incidents of illness involving histamine or suspected histamine in cheese were first reported to the FSA in 2003. Between 2001 and 2007, there were two reported incidents to the FSA linked to histamine in cheese; between 2008 and 2015, there were twenty such reported incidents (to note these data are provisional).

The risk based control of biogenic amine formation in fermented foods was comprehensively reviewed by EFSA in 2011. More recently, histamine in cheese was discussed at a meeting of the Committee on Toxicology of Food, Consumer Products and The Environment (COT) in June 2015. Given that there is a microbiological basis for the production of biogenic amines in cheese, the Agency would like to bring the issue to the Committee's attention and invite any observations or comments from the committee.

Detail**Formation of biogenic amines and associated problems**

1. Biogenic amines, such as histamine, are nitrogenous organic bases of low molecular weight. They may be classified as heterocyclic, aliphatic or aromatic according to their chemical structure, and divided into monoamines or diamines depending on the number of amine groups present. Histamine is classified as a heterocyclic diamine (EFSA, 2011)¹.
2. Formation of biogenic amines has been reported to occur in foods of animal origin having a high protein content, as well as in foods of plant origin. It can occur as a result of the spoilage microflora and/or intentionally added microorganisms, such as those used in the fermentation of foods. In food, biogenic amines are typically formed by the decarboxylation of amino acids. The primary biogenic amines present in food are histamine, tyramine, putrescine, cadaverine, and phenylethylamine; these are formed from the microbial decarboxylation of histidine, tyrosine, ornithine, lysine, and phenylalanine, respectively. Biogenic amines are thermostable and are not inactivated by heat treatments used in food processing or preparation such as cooking (EFSA, 2011). Higher amounts of certain amines may

¹ http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/2393.pdf

be found in foods as a consequence of the use of poor quality raw materials, microbial contamination, inappropriate conditions during food processing, and microbial contamination and inadequate conditions during storage.

3. Biogenic amine production in foods requires the availability of amino acids, the presence of microorganisms synthesising amino acid decarboxylases, and favourable conditions for their growth and decarboxylating activity. The amount and type of biogenic amines formed in foods is highly influenced by the intrinsic food characteristics including pH, water activity, composition, microbiological population and by extrinsic parameters such as storage time and temperature.
4. Cases of histamine poisoning typically occur following the consumption of spoiled or bacterially contaminated scombroid-type fish which can be fresh, frozen, or smoked fish, or canned fish products (EFSA, 2011). However, other foods such as cheese and wine have also been implicated in outbreaks of histamine poisoning. The consumption of food containing high amounts of toxic biogenic amine(s) may cause food intoxication or histamine poisoning with symptoms including flushing, headaches, nausea, cardiac palpitations, and increased or decreased blood pressure; in extreme cases the intoxication may be fatal (EFSA, 2011, Appendix A).
5. While histamine is the main focus of this paper, 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. However, it is only histamine incidents that have been reported to the Agency.

Conditions affecting histamine formation in foods

6. The conditions needed for histamine formation in food largely depend on the strains of decarboxylase-producing bacteria present, and can therefore vary markedly. Generally, biogenic amine formation rates increase with temperature and are lower at low temperatures due to the inhibition of bacterial growth, and a reduction in proteolytic and decarboxylase activities. The optimum temperature for biogenic amine formation has been reported to be between 20 and 37°C. While storage at low temperatures (<20°C) should reduce the degree of biogenic amine formation, some strains of bacteria have been found to actively contribute to biogenic amine accumulation at high rates during storage below 5°C (EFSA, 2011, Appendix A, TOX/2015/19, Appendix B).
7. A number of literature searches performed as part of EFSA's 2011 opinion on biogenic amines, acknowledged that, the optimum temperature for the formation of biogenic amines by mesophilic bacteria has been reported to be between 20 to 37 °C, while production of biogenic amines 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. *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. 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. Industry has raised concerns that efforts to reduce the salt content of cheese could lead to increased bacterial growth and therefore increased levels of histamine formation. EFSA stated that it can be assumed that the effect of salt either inhibiting or stimulating biogenic amine production is dependent on the strain of histamine-producing bacteria (EFSA, 2011, Appendix A; TOX/2015/19, Appendix B). Oxygen availability also appears to have a significant effect on the biosynthesis of biogenic amines. *Enterobacter cloacae* produces about half the quantity of putrescine under anaerobic compared with aerobic conditions, and *Klebsiella pneumoniae* synthesises significantly less cadaverine but acquires the ability to produce putrescine under anaerobic conditions (EFSA, 2011, Appendix A).

Micro-organisms associated with the formation of histamine and other biogenic amines in foods

8. Bacteria capable of producing biogenic amines possess biogenic amine biosynthesis pathways. A membrane antiporter protein has been identified as playing a key role in delivering the amino acid substrate into the cell for intracellular decarboxylation by amino acid decarboxylase. The antiporter protein is also responsible for removing the decarboxylated product (biogenic amine) from the cytoplasm into extracellular medium. (EFSA, 2011, Appendix A). An extensive literature search in EFSA's 2011 opinion identifies a number of bacterial biogenic amine/antiporter systems.
9. In microorganisms, two types of amino acid-decarboxylase enzymes have been described, having different physiological roles: biosynthetic (constitutive) mainly associated with microbial growth and biodegradative (inducible by a number of environmental factors) that fulfil a number of physiological roles depending on the microorganisms. The constitutive biosynthetic decarboxylases are present in far lower amounts than the induced biodegradative forms; the latter are responsible for biogenic amine accumulation in foods.
10. The physiological role of biogenic amine synthesis by biodegradative decarboxylases mainly appears to be related to protective mechanisms used by bacteria to withstand acidic environments. Decarboxylation increases survival under acidic stress conditions via the consumption of protons and the excretion of amines and carbon dioxide, helping to restore internal pH (EFSA, 2011, Appendix A).
11. **Histamine-** both Gram-negative and in Gram-positive bacteria are able to produce histamine. Many Gram-negative bacteria which commonly contaminate food are able to produce histamine. The strongest histamine producers *Hafnia alvei*, *Morganella morganii*, *Klebsiella pneumoniae* and, more recently, *Morganella psychrotolerans*, *Photobacterium phosphoreum*, *Photobacterium psychrotolerans* and have been isolated from fish incriminated in scombroid poisoning incidents (EFSA, 2011, Appendix A).

12. After fish, cheese (particularly ripening cheese) is the next most commonly implicated food item associated with histamine poisoning (EFSA, 2011, Appendix A). The formation of histamine in cheese, and some other fermented foods, is a simultaneous consequence of fermentation by lactic acid bacteria rather than spoilage due to bacterial contamination. In fermented foods, 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. It has also been suggested that histamine formation by bacterial decarboxylases can continue even following bacterial autolysis (EFSA, 2011, Appendix A). Certain fungi have been reported as capable of producing histamine from dairy products (e.g. *Debaryomyces hansenii*, *Geotrichum candidum*) (Linares *et al.*, 2012²).
13. **Tyramine-** in EFSA's 2011 opinion on biogenic amines, a number of micro-organisms capable of producing a variety of biogenic amines were also reviewed via extensive literature searching. 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*), *Leuconostoc* and *Lactococcus* and *Carnobacterium* spp. Bacteria within the genus *Staphylococcus* may also have a role in the production of tyramine. In fermented beverages, *L. brevis*, *L. hilgardii*, *Lactobacillus plantarum* and *Leuconostoc* spp. have been described as tyramine producers. Certain fungi have been reported as capable of producing tyramine from dairy products (e.g. *Yarrowia lipolytica*) (Linares *et al.*, 2012).
14. **Phenylethylamine, Putrescine and Cadaverine-** Phenylethylamine production is usually associated with tyramine production as demonstrated for *Enterococcus*, *Lactobacillus curvatus*, *Staphylococcus*. Putrescine and cadaverine production has mainly been related to Gram negative bacteria, especially in the families Enterobacteriaceae, Pseudomonadaceae and Shewanellaceae, generally associated with spoilage. Enterobacteria genera *Citrobacter*, *Klebsiella*, *Escherichia*, *Proteus*, *Salmonella* and *Shigella* are associated with production of considerable amounts of putrescine and cadaverine in food. Putrescine is one of the most common biogenic amines found in fermented products. Lactic acid bacteria, largely lactobacilli but also staphylococci have also been reported to be able to produce putrescine and/or cadaverine. Certain fungi have been reported as capable of producing putrescine (e.g. *Debaryomyces hansenii*, *Yarrowia lipolytica*) and cadaverine (*Yarrowia lipolytica* from dairy products (Linares *et al.*, 2012).

Histamine levels in cheese and limiting histamine production

15. Histamine levels in cheeses vary considerably; (TOX//2015/19 Appendix B, Table 4) illustrates clearly the histamine levels associated with a large variety of different cheeses and highlights the extent of variability in histamine and total biogenic amine content. Some of the highest histamine levels were found in a Swiss

² <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3390585>

(American) cheese (1470 – 5630 mg/kg) and a hard cheese Almkäse (1159.7 mg/kg). Even within a particular cheese variety it is evident that histamine levels can vary considerably. Factors such as long maturation periods and the use of unpasteurised milk, acid curds, or starter cultures that contain microorganisms known to produce histamine can produce higher histamine levels within cheeses, but not always. Bacterial “hot spots” have also been shown to occur in fish and histamine levels within these “hot spots” can be higher, highlighting that histamine is not necessarily distributed evenly throughout foods; whether such “hot spots” occur in cheese is unknown. The FSA understands that some of the larger cheese manufacturers and supermarket chains have set rejection limits of 500 mg/kg for histamine in cheese. The implementation of limits or monitoring such as this is less likely to be undertaken by smaller cheese manufacturers as testing for histamine is relatively expensive. The Agency anticipates that more details on histamine rejection limits and monitoring practices in the industry will be available at a later date as part of consideration of the issue with the COT (TOX/2015/19, Appendix B).

16. In 2011, EFSA’s BIOHAZ panel concluded that accumulation of biogenic amines in fermented foods is a complex process affected by multiple factors and their interactions, the combinations of which are numerous, variable and product-specific. Therefore, risk mitigation options, which are based on controlling those factors/interactions, cannot therefore be considered and ranked individually but considered in the context of general principles. Minimising the occurrence of biogenic amine-producing microorganisms can be achieved through ensuring that a good hygiene status of the raw materials is maintained and, where possible, additional microbial controls are employed. The panel also recommended that microorganisms intended to be used as starter cultures in any fermented food should be confirmed as not being biogenic amine producers and able to outgrow autochthonous microbiota under conditions of production and storage. The panel viewed that 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 the potential enhancing effects on biogenic amine formation and to enable dominance of starter culture(s) where used.

Histamine poisoning linked to cheese

17. Amongst the biogenic amines, histamine has attracted particular attention, as it has been identified or implicated as the causative agent in outbreaks of food poisoning involving scombroid fish and to a lesser extent cheese. Reported incidents of histamine poisoning in the literature include cheeses made from raw as well as pasteurised milk. The histamine concentrations in some cheeses that were implicated incidents were reported to range between 850 and 1870 mg/kg (EFSA, 2011). There is a perception by industry that incidents involving histamine in cheese are becoming increasingly common although the evidence supporting this is somewhat patchy. . Between 2001 and 2007, there were two reported incidents to the FSA linked to histamine in cheese; between 2008 and 2015, there were twenty such reported incidents (to note these data are provisional and provided in **Appendix C, for Members Use Only**). Incidents reporting high levels of histamine in fish have been ongoing. We are not aware of any incidents prior to

2003 including before the FSA was formed. The increased incident reporting may be as a result of an increase in the consumption of cheeses containing high levels of histamine, a general increase in the histamine levels of cheeses or an increase in awareness of the symptoms of histamine poisoning (TOX/2015/19, Appendix B).

18. Incidents reported to the FSA involving histamine in cheese appear to be more frequent than 10 years ago. Appendix C (For Members use only) provides further details concerning incidents involving histamine in cheese dealt with by the Food Standards Agency since 2001. From this information, it can be seen that the cheeses involved were largely mature cheddar cheeses. It is unclear why incidents tend to be associated with cheddar but may reflect a greater consumption of this type of cheese by children in nursery /school settings compared to other types of cheese. The incidents reported to the FSA typically involve children aged approximately 5 years of age, having consumed cheddar type cheeses in the form of a meal (e.g. lasagne or macaroni cheese) whilst at nursery or school. Some of these incidents have also involved adults (e.g. nursery staff) who have reported similar but milder symptoms after consuming or handling the implicated cheese. The data suggests that children may be particularly sensitive to high levels of histamine. Whilst the exact reason why young children are more sensitive to histamine is not clear, it has been proposed that their lower body weight may be a significant factor. However, the incidents data may be misleading due to the fact that reports of histamine poisoning are more likely to be made if multiple individuals are affected at the same time (i.e. a class of children) (TOX/2015/19, Appendix B). There is little or no information concerning sporadic cases of histamine poisoning and source involved ie scombroid fish, cheese or other foods.

Toxicological aspects

19. 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. The limited published information available allowed EFSA's BIOHAZ panel to suggest a potential acute reference dose (ARfD) of 50 mg of histamine per meal, per healthy adult³. This was discussed at a meeting of the Committee on Toxicology of Chemicals in Food, Consumer products and the Environment (COT) in June 2015 and considered to be sensible and conservative. When providing chemical risk assessment advice on histamine-related food incidents, the approach used by the Agency is that 200 mg/kg is an approximate threshold, below which, toxicity would not be expected. At 1000 mg/kg or above, toxicity would be expected. Effects at levels between these concentrations would depend on the amount consumed, individual susceptibility etc. The approach has been deemed appropriate by the COT:

³ The acute reference dose is an estimate of the amount of a substance (expressed on a body weight basis) that can be ingested over a short period of time, usually during one meal or one day, without appreciable health risk to the consumer.

Other biogenic amines

20. EFSA's BIOHAZ panel also examined the toxicological aspects of other biogenic amines in 2011, which are worthy of note. For tyramine, there is currently insufficient information related to establishing a No Observable Adverse Effects Level (NOAEL) in humans. Based on limited published information, no adverse health effects have been observed in healthy individuals not taking monoamine oxidase inhibitor (MAOI) drugs⁴ 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, currently 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.

21. The BIOHAZ Panel in 2011 recommended that further research is needed on: the toxicity and associated concentrations of histamine and tyramine in different foods. The panel also considered that there was a need to examine consumption data for fermented foods, especially cheese and the production process-based control measures for biogenic amines in fermented food production.

The Committee is asked:

- a) To note the reports of histamine poisoning associated with cheese and comment on the issue.

**Secretariat
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⁴ Administration of MAOI drugs is known to increase an individual's sensitivity to tyramine by decreasing its breakdown; this can result in severe headaches when sources of tyramine (such as cheese) are consumed, and is often referred to as the 'cheese reaction'.

Appendices:

- **Appendix A** – EFSA opinion on biogenic amines (hard copy on request) 2011
http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/2393.pdf
- **Appendix B** – COT paper on histamine in cheese (TOX/2015/19)
- **Appendix C (Members only)** - Summary table of FSA incidents involving histamine since 2001.