Opinion of the Scientific Panel on Biological Hazards on the request from the Commission related to the effects of Nitrites/Nitrates on the Microbiological Safety of Meat Products. [1]

(Question N° EFSA-Q-2003-026)

Adopted on 26 November 2003

SUMMARY

By definition, "cured meat products" contain curing salts, usually salt (sodium chloride) and either nitrites or nitrates. The use of nitrites as a curing agent provides the cured meat colour, the cured meat flavour, and, in combination with other factors, slows or prevents growth of bacterial pathogens.

Nitrites exert a concentration-dependent antimicrobial effect in cured meat products, including inhibition of the outgrowth of spores of putrefactive and pathogenic bacteria such as *Clostridium botulinum*. Their antimicrobial effects are pH-dependent, increasing ten-fold for each unit fall in pH. In most cured meat products, the addition of nitrites (or nitrates) is necessary to prevent the growth and toxin production by *C. botulinum*.

The extent of protection provided to cured meats against microbial growth has been attributed by different researchers to many factors including the input concentration of nitrite, the residual nitrite concentration, the salt concentration of the product, the addition of sodium ascorbate (or isoascorbate / erythorbate), the heat treatment applied, the storage temperature, the initial pH of the meat, and the spore load initially present. The extent of protection is due to a combination of factors rather than any single factor.

The antimicrobial effect of nitrite is also markedly influenced by the presence of iron. The availability of iron forms capable of binding and inactivating nitrite varies with the type of animal tissue processed (e.g. it is high in liver and blood sausage, lower in beef, while pork and chicken have the lowest iron content). The absence of a protective effect of nitrite in liver sausages has been ascribed to the presence of high natural quantities of iron in the product.

Despite the vast amount of research, there is still no universally accepted explanation of the control of *C. botulinum* in meat products. An obvious difference between commerce and

[1] For citation purposes: Opinion of the Scientific Panel on Biological Hazards on a request from the Commission related to the effects of Nitrites/Nitrates on the Microbiological Safety of Meat Products, *The EFSA Journal* (2003) 14, 1-34

laboratory investigations is the number of spores of *C. botulinum* present initially. In the inoculum of experimental studies the number of spores present is several orders of magnitude greater than their relatively rare occurrence in commercial products. This is important because in laboratory experiments there is consistently less growth and less toxin production with a low inoculum than with a high inoculum.

There is no direct relationship between the input nitrite and the residual nitrite, especially if sodium ascorbate (or sodium isoascorbate / sodium erythorbate) is present in the formulation to prevent oxidation and to fix the colour.

The Panel is of the opinion that the ingoing amount of nitrite, rather than the residual amount, contributes to the inhibitory activity against *C. botulinum.* Therefore, control of nitrite in cured meat products should be via the input levels rather than the residual amounts.

The amount of nitrite necessary to inhibit *C. botulinum* differs from product to product. With good hygiene, HACCP and realistically short storage times under good temperature control, some meat products can be produced without using nitrites, although these are not strictly "cured meat products". The practices mentioned above are essential whenever levels of nitrites are reduced.

In other products, especially those with a low salt content and having a prolonged shelf-life, addition of between 50-150 mg/kg nitrite is necessary to inhibit the growth of *C. botulinum*.

The Panel is of the opinion that "indicative ingoing amount" nitrates and nitrites be "maximum ingoing amount".

Substantial research efforts failed to identify an alternative to sodium nitrite for the production of cured products.

Nitrates have no direct activity against *C. botulinum*. In particular traditional products (e.g. dry-ripened fermented sausages of high pH, dry cured ham), nitrates act as reservoirs of nitrites, generated by microbial activity.

The carcinogenic effects of nitrosamines, the result of transformation of nitrites and nitrites, are outside the scope of this opinion.

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BACKGROUND

Nitrites and nitrates are currently authorised as additives in annex III part C to European Parliament and Council Directive 95/2/EC on food additives other than colours and sweeteners[2].

Potassium and sodium nitrite (E 249 and E 250) are authorised for use in the following foodstuffs. Residual amounts are fixed for all foodstuffs, as well as indicative in-going amounts for some of them.

E No	Name	Foodstuff	Indicative ingoing amount	Residual amount
			mg/kg	
E 249 E 250	Potassium nitrite (1) Sodium nitrite (1)	Non-heat-treated, cured, dried meat products	150 (2)	50 (3)
		Other cured meat products Canned meat products *Foie gras, foie gras entier, blocs de foie gras*	150 (2)	100 (3)
		Cured bacon		175 (3)

Sodium and potassium nitrate (E 251 and E 252) are authorised for use in the following foodstuffs. Residual amounts are fixed for all foodstuffs, as well as indicative in-going amounts for some of them.

E No	Name	Foodstuff	Indicative ingoing amount	Residual amount
E 251 E 252	Sodium nitrate Potassium nitrate	Cured meat products Canned meat products	300	250 (4)
		Foie gras, foie gras entier, blocs de foie gras		50 (4)
		Hard, semi-hard and semi-soft cheese Dairy-based cheese analogue		50 (4)
		Pickled herring and sprat		200 (5)

2 OJ L 61, 18.3.1995, p.1

(1) When labelled 'for food use', nitrite may only be sold in a mixture with salt or a salt substitute.
(2) Expressed as $NaNO_2$.
(3) Residual amount at point of sale to the final consumer, expressed as $NaNO_2$.
(4) Expressed as $NaNO_3$.
(5) Residual amount, nitrite formed from nitrate included, expressed as $NaNO_2$.

When implementing Directive 95/2/EC and the above levels, Denmark notified the Commission in 1996 of the levels already fixed in its national legislation and asked to keep these national provisions as derogation from Directive 95/2/EC. In 1999, the European Commission adopted a Decision (1999/830/EC[3]) rejecting the Danish request. Denmark challenged this Decision in the European Court of Justice (case C-3/00).

On 20 March 2003, the European Court of Justice delivered its judgement in the case C-3/00. This judgement has annulled Commission Decision 1999/830/EC with regard to the national provisions notified by Denmark concerning the use of nitrites and nitrates in foodstuffs.

In its findings, the Court states that the Commission Decision did not take sufficient account of the 1995 opinion of the Scientific Committee on Food (SCF) on nitrites and nitrates. According to the Court, the Commission failed to mention in this connection that the maximum amounts of nitrites set in Directive 95/2/EC are called into question by the SCF opinion. The SCF noted that the residual amounts of nitrites permitted by the Directive were higher than those expected if the recommended amount of nitrites added was respected.

In fact the Directive fixes indicative in-going amounts which correspond roughly to the ones indicated by the SCF, but the Directive fixes mandatory residual amounts and it is these residual amounts which were questioned by the SCF.

For the Court it followed that, since the Commission failed duly to take into account the SCF opinion in assessing the justification for the Danish provisions concerning the use of nitrites and nitrates, its Decision is vitiated by a defect, which renders it unlawful.

The findings of the Court concerning the opinion of the SCF and concerning the provisions on nitrates and nitrites in Directive 95/2/EC require follow-up.

From a technical point of view, the addition of nitrites and nitrates to foodstuffs reinforces the preserving effect of smoking, salting or cooking, for example in meat products. On one hand, these substances inhibit the growth of bacteria, which can cause the deterioration of foodstuffs, as well as that of pathogenic bacteria such as *Clostridium botulinum*, which causes botulism in humans. On the other hand, in meat products, nitrites and nitrates are transformed into nitrosamines[4], which are recognised carcinogens.

In its opinions dated from 1990 and 1995, the SCF recommended that "exposure to preformed nitrosamines in foods should be minimised by […] lowering the nitrite addition to foods to the minimum necessary to achieve the required preservative effect and ensure microbiological safety". The Committee however did not indicate what these levels would be.

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[3] OJ L 329, 22.12.1999, p.1

[4] this statement is incorrect and should read: a proportion of nitrites may be transformed into nitrosamines (quotation from EFSA)

Therefore, the Commission wishes to find a balance between keeping the level of nitrosamines as low as possible by lowering the levels of nitrites and nitrates added to food and ensuring the microbiological safety of the food.

TERMS OF REFERENCE

The Commission asks the European Food Safety Authority to deliver an opinion on the effect of nitrites and on the microbiological safety of meat products, in particular related to *Clostridium botulinum*.

In doing so, the Authority is asked to address and answer the following questions:

(1) What is the correlation between in-going and residual amounts of nitrites and nitrates?

(2) What is the effect of the in-going and residual amount of nitrites and nitrates on the microbiological safety of meat products, in particular related to *Clostridium botulinum?*

(3) Which is the lowest level of in-going or residual amount of nitrite and nitrates, which would still have a protective effect in meat products against microbiological risks, in particular related to *Clostridium botulinum*?

To complete the picture, the effect of nitrites and nitrates on shelf life and organoleptic properties should be dealt with to some extent, as well as the use of possible alternatives for the use of these substances as preservatives in meat products.

ASSESSMENT

1. INTRODUCTION

Meat is an ideal medium for the growth of many organisms because it is high in moisture, rich in proteins, peptides and amino acids and plentifully supplied with minerals and accessory growth factors. In addition it has some fermentable carbohydrates, usually glycogen, and has a favourable growth pH for multiplication of most micro-organisms. Consequently, meat and meat products are extremely perishable unless appropriately preserved and/or stored under conditions designed to retard microbial activity and proliferation.

1.1. Origin of micro-organisms in meat

With the exception of external surfaces of the animal and the gastro-intestinal and respiratory tracts, the musculature of healthy living animals is free of micro-organisms (Mackey and Derrick, 1979). Once the animal is slaughtered, however, the internal defence mechanisms that combat infectious agents in the living body are lost. Thus, from the point of exsanguination, and at every stage thereafter, measures must be taken to ensure the minimization of microbial contamination and proliferation (Ingram, 1962).

On meat carcasses dressed under good hygiene, the majority of the bacteria originate from the hide. Errors during dressing can lead to contamination from the intestinal tract of the animal. Other microbial contaminants can come from the floors, contact surfaces, knives, hands, etc. Hence, any careless slaughter procedures contribute to contamination of the carcass. Contamination during slaughtering occurs during removal of the hide/fleece of cattle/sheep, during dehairing of pig-skin, and during evisceration (Grau, 1986). As a consequence many types of micro-organisms are present on carcasses and the meat products thereof. Among these many ubiquitous organisms, *Clostridium botulinum* will inevitably be present from time to time. An overview of the presence of *C. botulinum* in meat products is presented in Table 1. Clostridia, and occasionally *C. botulinum*, are often present in the liver of healthy slaughtered animals (Avery et al., 1959; Canada and Strong, 1964; Kerry, 1964; Muller, 1967; Roberts et al., 1970; Hauschild and Hilsheimer, 1983).

Table 1. Presence of *C. botulinum* in meat products (Roberts and Smart, 1976)

Food	Sample weight (g)	No of samples	Total weight of samples (kg)	No of samples containing *C. botulinum*	Spores per kg*	Ref.
Vacuum-packed bacon	25	263	6.575	11	2.17**	(1)
Vacuum-packed bacon	50	108	5.4	6		
Vacuum-packed bacon	175	26	4.55	19		
Cooked ham	30	100	3.0	5	1.66	(2)
Smoked turkey	30	41	1.23	1	0.81	(2)
Vacuum-packed frankfurters	150	10	1.5	1	0.66	(3)
Luncheon meat	12 x 2g	73	1.75	1	0.57	(4)
Chicken	6 x 0.5g	1078	3.23	1	0.31	(5)

*assuming one spore only in each sample containing *C. botulinum*.
** calculated across all samples of vacuum-packed bacon, reference (1).

(1) Roberts and Smart ,1976). (2.) Abrahamsson and Riemann ,1971. (3) Insalata et al.,1969. (4) Taclindo et al., 1967. (5) Greenberg et al.,1966.

1.2. Preservation

Microbial growth in meat is determined by intrinsic factors such as pH, water activity (a_W), oxidation-reduction potential, available nutrients, and extrinsic factors such as temperature, presence or absence of oxygen. It is widely appreciated that temperatures below about 4-8°C retard the proliferation of many spoilage organisms and also prevent growth of many pathogenic bacteria.

For extended storage of meat, preservation is necessary. Important methods include heating, drying, fermentation and the use of preservative agents, but their use may be limited by their effect on the food. For example, the quantity of salt as preservative is limited by sensory acceptability, while heating is limited by loss of eating quality. To maintain the quality of meat products, a combination of preservation methods is often used, such as a mild heat treatment and the use of curing salts.

1.3. *Clostridium botulinum* and meat

As indicated above, no raw meat product is completely sterile and the potential always exists for the presence of pathogenic micro-organisms able to give rise to infections and intoxications. Foodborne infection is an illness caused by ingestion of pathogenic organisms that proliferate and cause illness in the host. Foodborne intoxication is an illness caused by ingestion of a toxin that has been pre-formed by a pathogenic organism in the food itself, or in response to a toxin that is released during multiplication of the pathogen in the gastro-intestinal tract. Among intoxications, the pre-formed neurotoxins produced by *C. botulinum* are of particular concern because of the high mortality rate and long periods required for recovery from intoxication. Because spores of *C. botulinum* occur in soil all over the world, it must always be regarded as a potential meat-borne hazard. Those spores survive many heat treatments used in food processing, and, given the right conditions, can develop and grow in many foods. Unless processors take preventive measures to inactivate *C. botulinum*, or to inhibit its growth and toxin production, botulism outbreaks could occur.

Foodborne botulism results from consumption of food in which *C. botulinum* has multiplied and produced toxin. The botulinum neurotoxins are produced in vegetative cells after spores have germinated, and toxin production is strongly related to growth. The botulinum neurotoxins are proteins that are elaborated during multiplication and when the vegetative cell lyses. After ingestion of the food that contains toxin, toxin is absorbed in the intestinal tract and binds irreversibly to the peripheral nerve endings where it inhibits the release of neurotransmitters. Signs and symptoms of botulinum intoxication comprise nausea, vomiting, fatigue, dizziness, dryness of mouth and throat, constipation, paralysis of the muscles, blurred and double vision and difficulties in respiration. These symptoms may develop within 12-72 hours after consumption of the toxin-containing food. Treatment includes administration of botulinum antitoxin and appropriate supportive respiratory assistance. If death does not occur, recovery often takes several months. Today the mortality is around 10 %, mainly because respiration can be maintained artificially. Tables 2a and 2b present an overview of reported cases of botulism. Lund and Peck (2000) provide more detail of the presence of *C. botulinum* in foods and approaches to controlling its growth and toxin production.

Table 2a. An overview of reported outbreaks[1] of botulism with meat and fish implicated (Hauschild, 1993).

Country	Period	Reported outbreaks with food identified	Food (%)		Food source (%)	
			Meats	Fish	Home	Commerce
United States	1971-1989	222	16	17	92	8
Canada	1971-1989	75	72	20	96	4
Poland	1984-1987	1500[2]	83	12	75	25
Hungary	1985-1989	28	89	0	100	0
France	1978-1989	123	89	3	62	38
Spain	1969-1988	48	38	2	90	10
Denmark	1984-1989	10	100		100	0
Norway	1961-1990	19	16	84	100	0

[1] One or more cases caused by one type of food (following the definition of botulism outbreak of the WHO Surveillance Programme).
[2] Number of cases.

Table 2b. An overview of reported outbreaks[1] of botulism in countries participating in the WHO Surveillance Programme for Control of Foodborne Infections and Intoxications in Europe (2001)

Selected countries	Year					
	1993	1994	1995	1996	1997	1998
Russian Federation	521	577	645	518	491	502
Poland	143	116	118	107	81	93
Uzbekistan	98	71	135	145	106	96
France	20	36	28	8	23	29
Germany	17	13	12	15	7	21
Norway	0	1	1	0	8	6
Denmark	0	0	2	0	0	1
All countries[2)]	1,182	1,331	1,348	1,252	1,160	1,351

[1)] One or more cases caused by one type of food (following the definition of botulism outbreak of the WHO Surveillance Programme).
[2] "All countries" = all countries participating in the WHO Surveillance Programme.

From the data in Table 2a it is clear that meat products are a major cause of foodborne botulism in some countries. In the majority of cases home-preserved products were implicated. In Table 2b, reported outbreaks of botulism in European countries illustrate that the number of cases remained relatively constant in the period 1993 through 1998.

C. botulinum originates in the soil and is carried into the food chain on and in the live animal. Good hygienic practices at slaughter minimise the chances that C. botulinum is present on the carcass, although its complete absence from meat is impossible to achieve with current technologies. Conditions that favour growth and toxin production by C. botulinum include relatively high moisture, low salt, low acid (i.e., pH > 4.6), food stored under restricted oxygen conditions, and a temperature above the minimum for growth (viz 3°C for non-proteolytic strains and 10°C for proteolytic strains). In Table 3, conditions limiting growth are summarised, together with the heat resistance of spores.

Unfortunately, meat is an excellent medium for the growth of C. botulinum and toxin production. Many publications confirm the efficacy of sodium nitrite in inhibiting growth and toxin production by C. botulinum in perishable cured meats such as wieners, bacon, canned ham, luncheon meat and comminuted products. Safety, however, cannot be totally attributed to nitrites alone, but rather to a number of factors acting in combination, such as heat treatment, pH, salt (a_w), redox potential, and the initial numbers of bacterial spores, normally low, in the meat and other ingredients. Other agents, such as ascorbate or isoascorbate, have also been reported to influence the efficacy of nitrite. One of the purposes of using ascorbate is that, in concentrations equimolar with nitrite, the formation of nitrosamines is reduced (Mottram et al., 1975).

Table 3. Minimal requirements for growth and heat resistance of *C. botulinum* types A, B, E and F and heat resistance of the spores (from various sources)

Properties	Group	
	Proteolytic strains	Non-proteolytic strains
Toxin types	A, B, F	B, E, F
Inhibitory pH	4.6	5.0
Inhibitory NaCl concentrations	10%	5%
Minimal a_W for growth	0.94	0.97
Temperature range for growth	10 - 48° C	3 – 35° C
D_{100}* of spores	25 min	< 0.1 min

* Time at 100°C to reduce viable numbers to 10% of the initial number

1.4. Inhibitory properties of nitrite/nitrate

In medieval times, treating meat with salt containing nitrate (saltpetre) was a commonplace method of preservation. During the 1920s, when investigations on the curing effects of both nitrate and nitrite commenced, it was observed for the first time that nitrate exerted no special restrictive effects on bacterial activity in neutral or alkaline conditions. In acid solutions, however, marked inhibition of bacterial growth was evident (McNeal and Kerr, 1929). The authors attributed this inhibitory activity to the production of small amounts of nitric and nitrous acids in mixtures containing reducing substances. Later, Tarr (1941, 1942) reported the bacteriostatic action of sodium nitrite against a number of bacterial species present in fish muscle. Tarr (1941) also demonstrated the importance of pH to the efficacy of nitrites. For example, at pH 7.0 little or no inhibition was observed, unlike at pH 5.7 and 6.0 when microbial growth was inhibited. Even in 1950, the relative roles of nitrates and nitrites as preservatives in cured meats were still unclear. The disappearance of nitrite during processing and storage was assumed to make it an unreliable preservative. Nevertheless, the concept of a combined effect of heating in the presence of curing salts was proposed. Although thermal destruction of bacterial spores was not enhanced by the presence of curing salts, there was an increased inhibition of outgrowth by salt, and to some degree, by nitrite.

The following paragraphs are based on a comprehensive overview of research activities on the use of nitrite to preserve meat products by Tompkin (2004). During the 1950s the relative significance of salt, nitrite and nitrate as preservatives was somewhat clarified. Eddy and Ingram (1956), and many others, demonstrated that the use of nitrate did not result in stable commercial canned cured meats. A key study by Silliker et al. (1958), on shelf-stable canned cured luncheon meat, helped to establish the future use of nitrite by showing that nitrate played absolutely no role in retarding putrid spoilage i.e. multiplication of micro-organisms physiologically similar to proteolytic strains of *C. botulinum*, and even stimulated spoilage by aerobic spore forming organisms. The same authors demonstrated that addition of salt alone was not responsible for the stability of the product, and it was suggested that the key to stability was the addition of sodium nitrite together with heat injury to the relatively small number of spores present initially.

By the end of 1950s it was clear that nitrate has no antibacterial effect and that nitrite played a significant role in the stability of shelf-stable canned meats. It was suspected that nitrite prevented germination or outgrowth of surviving heat injured spores of *C. botulinum*. Brine content (% w/w NaCl on water) was further shown to influence spore germination, growth and toxin formation. The role of nitrite as a preservative in perishable cured meats was, however, still in doubt.

The use of nitrates appears necessary in particular traditional dry meat products, typical of the Mediterranean countries, like long-ripened dry-fermented sausages and dry-cured ham. Traditional dry-fermented sausages experience a long-ripening process (up to several months) with a slow and mild pH drop (usually the pH remains relatively high), where the added nitrate is progressively reduced to nitrite contributing not only to safety but also to specific and characteristic sensory properties. Dry-cured ham is an entire piece where curing salt has to be rubbed on the outer surface of the ham and left for several weeks for its diffusion through the entire piece (Toldrá, 2002). This is a very slow process that may take up to 2 months under cold storage, where the progressively

diffused nitrate constitutes a reservoir of nitrite. In this way, nitrite effectively reaches the centre of the ham; especially to critical inner locations like the bone joints, where protection by nitrite is essential before the next stages of processing, which increase the temperature for ripening and drying of the product.

During the 1960s, increased emphasis was placed on understanding the role of nitrite in cured meats and its effect on thermally injured spores. This was especially important as the increased use of vacuum packaging raised new questions regarding the microbiological safety of cured meats. Research in this area was fuelled by outbreaks of botulism arising from temperature abused vacuum-packed smoked fish products.

Progress during the 1960's toward defining the role of nitrite in cured meats included:

- a number of investigations confirmed the results obtained by Silliker *et al*., (1958);
- the concept of thermal injury to spores that survive the processing of shelf-stable canned cured meats was valid;
- factors recognised as influencing growth include pH, heat treatment, residual nitrite, storage temperature and number of spores present.

During the 1970s and 1980s, the occasional detection of N-nitrosamines in cured meats became of great concern. Throughout this period, pressure mounted to reduce nitrate and/or nitrite addition to meat products and even to eliminate them completely.

Research continued to examine the role of nitrite in perishable canned cured meats and to unravel the mechanism of the inhibitory efficacy of nitrite against formation of botulinum toxin. Pivnick and Chang (1974) claimed that the residual nitrite level was not the key antimicrobial fraction, and that the nitrite that was "lost" (i.e. analytically undetectable) to the meat system was more likely to be inhibitory to bacteria.

It was recognised that the safety of cured meats such as wieners, bacon, canned hams and luncheon meat was based on several factors, as described above. By the end of the 1970s it was generally accepted that the level of nitrite needed to control botulism, must be determined for each individual class of product. Tompkin et al. (1978a) and Christiansen (1980) argued that growth of *C. botulinum* was dependent on residual level of nitrite present. They hypothesised that if spores germinated while nitrite remained they failed to grow; but if germination was delayed until after all the residual nitrite was "used up", they were not inhibited and grew.

Factors, such as pH and ascorbate concentration, affect inhibition because they influence the rate of nitrite depletion (Tompkin et al., 1978b). It was shown that the addition of ascorbate can act in concert with residual nitrite to retard botulinum outgrowth in freshly produced bacon. The authors demonstrated that ascorbate, and also cysteine, enhanced the antibotulinum efficacy of nitrite in cured meat by sequestering metal ions in the meat rather than by an anti-oxidative or reducing mechanism. In this respect the iron contents of meat was recognized as important. Benedict (1980) reported that restriction of iron, through inhibition of solubilisation contributed to the inhibition of nitrite. It was also observed that in meats with naturally high levels of iron, e.g., liver and beef hearts, the

anti botulinum effect of nitrite was reduced (Tompkin et al., 1978b). Sequestering of iron by adding ascorbate or EDTA restored the inhibitory effect of nitrite. Reddy *et al*, (1983) concluded that nitric oxide inactivation of iron-sulphur proteins was the probable mechanism of inhibition of *C. botulinum* in nitrite-cured meats. It was also demonstrated that adding ascorbate causes a more rapid loss of residual nitrite, which, at the time, was considered a negative effect.

However, there is no convincing evidence that the residual amount of nitrite contributes to the microbiological safety of meat products. For example, in meat products containing ascorbate (or isoascorbate / erythorbate) the residual nitrite content is very low and sometimes below the level of detection, yet growth of *C. botulinum* is prevented.

Data on the effect of nitrite on other pathogenic micro-organisms such as *Staphylococcus aureus*, salmonellae and *Listeria monocytogenes* over the course of time were summarized by Tompkin (2004), who concluded that:

- nitrite can contribute to the inhibition of *L. monocytogenes* under certain circumstance e.g. refrigerated storage, but not all;
- nitrite is not effective in controlling Gram negative enteric pathogens in commercially prepared foods.

1.5. Other technological properties of nitrite

There are other roles of nitrite with technological significance in cured meats (Flores and Toldrá, 1993; Pegg and Shahidi, 2000):

a) Contribution to the colour in cured meats through the formation of nitrosylmyoglobin - upon heating, a myochromogen pigment is formed giving a characteristic pink colour to the cooked cured meat products.
b) Contribution to the oxidative stability of lipids where nitrite plays an antioxidant role through different mechanisms.
c) Contribution to the development of typical and distinctive cured meat flavour although the formation and identity of the volatile compounds responsible for the cured flavour is not yet fully understood (Ramarathnam, 1998).

2. THE EFFECTIVE CONCENTRATION OF NITRITE TO PREVENT OUTGROWTH OF SPORES OF *C. BOTULINUM*

Although the extent of protection provided by nitrite depends on several factors, many experiments have determined the minimum amount of nitrite that provides an inhibitory effect on germination and growth of *C. botulinum*. In the following sections / paragraphs a number of relevant experiments and results are described. Throughout the 1960s and 1970s nitrite was generally seen as undesirable, with major efforts to minimise its use in cured meats because of concerns regarding the formation of nitrosamines. More recently, there is evidence that ingested nitrate or nitrite, when acidified in the stomach, is anti-bacterial for gastrointestinal bacteria (Archer, 2002).

2.1. Control of non-proteolytic strains

Spores of non-proteolytic strains of *C. botulinum* are much more sensitive to heat than those of proteolytic strains (see Table 3). Consequently they are not a problem in shelf-stable canned meats. Heating at 90°C for at least 10 minutes is considered to inactivate spores to such an extent that no health risk remains. Such heat treatment is prescribed in many hygiene codes for so-called 'refrigerated, processed foods of extended durability' (REPFEDS). It has also been demonstrated that strains of non-proteolytic *C. botulinum* are unable to grow in a 4% brine solution (a_W <0.97) irrespective of pH and storage temperature.

Lücke et al. (1981) prepared bologna-type mixtures of a_W 0,977 – 0,985 and varying amounts of nitrite, inoculated them with spores of non-proteolytic *C. botulinum*, and cooked the products in cans to about 76°C core temperature. This process inactivated all vegetative cells and destroyed about 90% of spores added. The protective effect of the nitrite added is presented in Table 4. The protective effect is expressed as the probability that a given spore of *C. botulinum* survives the heat treatment and produces toxin in the product. For example, a protective effect of 6.0 means that only 1 spore per 10^6 spores present will survive and produce toxin (i.e. a total of 10^6 spores would have to be present in the product "batch" for one spore to produce toxin).

Table 4. Protective effect of in-going nitrite in artificial contaminated bologna-type sausages (3% brine; pH 6.0) heated until a core temperature of about 76 °C (Lücke and Roberts, 1993).

Storage temperature (°C)	Protection[1] from toxin formation by non-proteolytic *C. botulinum* with indicated in-going amounts of $NaNO_2$			
	0 mg/kg	40 mg/kg	62 mg/kg	83 mg/kg
15	< 4.5	< 4.5	< 4.5	< 4.5
10	< 4.4	4.6	5.2	6.2
8	4.6	4.9	6.4	6.0
5	6.8	7.3	> 7.3	> 8.0

[1] Protection is defined as log (1/P) where P is the probability that a given spore survives the heat treatment and produces toxin in the product) For example, a protective effect of 6.0 means that only 1 spore per 10^6 spores present will survive and produce toxin.

In the bologna-type sausages with no added nitrite growth was observed after 1, 2 and 3 weeks at 15, 10 and 8°C, respectively, and some cans became toxic after about 16 weeks at 5°C. Nitrite addition gave significantly better protection against toxin formation.

Identical experiments were carried out with liver sausage mixtures, but different amounts of added nitrite, showed no beneficial effects.

2.2. Control of proteolytic strains

Spores of proteolytic strains of *C. botulinum* are much more heat resistant than spores of non-proteolytic strains and germinate and proliferate at $a_W > 0.94$ (10% NaCl) and a pH > 4.6. Based on these characteristics, spores of proteolytic strains are of greater concern than those of non-proteolytic strains.

In many meat products, complete destruction of spores of proteolytic strains of *C. botulinum*, as a measure to prevent botulism, is not an option, because a 'botulinum cook'[5] (Fo = 3) of sausages or other salted and/or cured meat causes undesirable changes in appearance and flavour. To avoid such undesirable deterioration, meat processors generally cook their shelf-stable salted and/or cured products only to Fo values between 0.1 and 1.5. Such heat treatments reduce the number of viable spores of proteolytic types of *C. botulinum* by about 1 to 7 $\log_{10}$ cycles. As a consequence, this lower heat process must be compensated for by inhibiting any surviving spores by intrinsic factors, including NaCl (low a_W), low pH and addition of nitrite. However, it must be noted that the pH of meat is difficult to control, or even make allowance for, other than by selection of meat with low natural pH e.g. < 6.0.

The effects of all combinations of several factors listed below, previously reported to influence toxin production by proteolytic strains of *C. botulinum*, were described by Roberts et al. (1981a, b, c; 1982), Robinson et al. (1982) and Gibson et al. (1984), using a pork slurry system:

- NaCl (% on water) 2.5, 3.5, 4.5
- the level of added nitrite (mg/kg) 100, 200, 300
- the effect of chemical additives *viz* sodium nitrate (0 and 500 mg/kg); sodium ascorbate (0 and 1,000 mg/kg); a polyphosphate commonly used in cured meats (0 and 0.3%); and potassium sorbate (0 and 0.26%) as a possible partial replacement for nitrite;
- incubation time up to 6 months
- incubation temperature (°C) 15, 17.5, 20, 35
- unheated and two heat treatments representative of cured meat cooking;
- the (natural) pH of the slurry ("low" 5.5-6.3 and "high" 6.3-6.8)
- the inoculum size (10 or 1,000 spores per 28 g sample).

[5] The "botulinum cook" is defined as equivalent to 3 min. heating at 121°C. This value is also called the F_o value or the "process value". The F_o value required for canned food product is equivalent to 12-decimal reductions of *Clostridium botulinum* spores. Using the highest known D-values (0.25 min at 121°C) the F_o is therefore equal to 12 x 0.25 = 3. This is the so-called 12 D-concept designed to reduce the bacterial load of one billion spores in each of 1000 cans to one spore in a thousand cans.

After analysing the data by several methods, results were expressed as the probability of toxin production.

Robinson *et al*. (1982) tested the effect of the natural pH of meat (pH 5.5-6.3 and pH 6.3-6.8) and nitrite on growth and toxin production by proteolytic *C. botulinum* in a ground pork slurry system by adding 100, 200 and 300 mg nitrite per kg. The products were inoculated with spores of *C. botulinum* types A and B, packed anaerobically and heat treated either at 80 °C for 7 minutes (low heat treatment) or at 80 °C for 7 minutes plus 80 °C for 1 hour (high heat treatment) and incubated the samples at 15, 17.5, 20 and 30°C. Some examples of probabilities of toxin production by proteolytic *C. botulinum* are listed in Table 5.

Table 5. The effect of in-going $NaNO_2$ and (natural) pH on toxin production by proteolytic *C. botulinum* type A and B in pork slurry system (2.5% NaCl, inoculum 10 spores/28g) (data from Robinson et al, 1982)

Treatment	Low heat treatment		High heat treatment	
	pH		pH	
	6.0	6.5	6.0	6.5
$NaNO_2$ 100 mg/kg*	76**	96	59	86
$NaNO_2$ 200 mg/kg	25	79	13	57
$NaNO_2$ 300 mg/kg	3	35	1	23

* nitrite input
**probability (%) of toxin production

The results demonstrate that the pH of the meat, the heat treatment, and the concentration of nitrite all affect growth and toxin production by *C. botulinum.*

Robinson *et al*. (1982) also tested the effect of nitrite on growth and toxin production by proteolytic *C. botulinum* in the ground pork slurry system described above, by adding 100, 200 and 300 mg nitrite per kg with and without the addition of 1000 mg ascorbate per kg. Representative results are presented in Table 6.

In the presence of 100, 200 or 300 mg $NaNO_2$ /kg product the probability of toxin production decreased with increasing nitrite from 96% to 35% for low heated slurry, and from 86% to 23% in the high heated slurry. In the presence of ascorbate, the probability of toxin production in the low heated slurry was 26% to 1% and 8% to 0% in the high heated slurry.

The effect of initial numbers of *C. botulinum* spores on the inhibitory effect of nitrite was also tested by Robinson et al. (1982) using the pork slurry system with inoculum sizes of 10 and 1000 spores per 28 g of slurry and $NaNO_2$ 100, 200 and 300 mg/kg.. Results for 15 and 20 °C are presented in Table 7.

Table 6. The effect of in-going $NaNO_2$ and ascorbate on toxin production by proteolytic *C. botulinum* type A and B in a pork slurry system (pH 6.5, 2.5% NaCl, inoculum 10 spores/28g) (data from Robinson et al, 1982)

Treatment	Low heat treatment	High heat treatment
$NaNO_2$ 100 mg/kg*	96**	86
$NaNO_2$ 100 mg/kg + 1000mg/kg ascorbate	26	8
$NaNO_2$ 200 mg/kg	79	57
$NaNO_2$ 200 mg/kg + 1000mg/kg ascorbate	5	2
$NaNO_2$ 300 mg/kg	35	23
$NaNO_2$ 300 mg/kg + 1000mg/kg ascorbate	1	0

* nitrite input
** probability (%) of toxin production

Table 7. The effect of inoculum size of proteolytic *C. botulinum* type A and B in pork slurry system (pH 6.5, 2.5% NaCl) (data from Robinson et al, 1982)

Treatment	Inoculum (per 28 g)			
	1000		10	
	Incubation temp.		Incubation temp.	
	15 °C	20 °C	15°C	20 °C
$NaNO_2$ 100 mg/kg*	71**	91	27	59
$NaNO_2$ 200 mg/kg	20	50	4	13
$NaNO_2$ 300 mg/kg	2	9	0	1

* nitrite input
**probability (%) of toxin production

The results demonstrate that the number of spores present initially strongly influences the probability of growth and toxin production, confirming indications in the 1960's (see paragraph 1.4) Even the lower inoculum was appreciably higher than levels of *C. botulinum* spores occurring naturally – 10 spores per 28 g is equivalent to 350 spores per kg, compared with not more than 2 per kg detected in a range of meat products (Table 1).

It is also clear that the protective effect increases with increasing concentrations of added nitrite.

Lücke and Hechelmann (1986) studied the microbiological stability of various commercial and experimentally inoculated products during prolonged storage without refrigeration. Table 8 shows that common formulations and processes for German-type cured meat products, cooked in hermetically sealed containers, gave a protection[6] between 2.3 and 6.3 if no nitrite was added.

Addition of nitrite improved the protection considerably in case of "Brühwurst" (see Table 8). However, addition of nitrite at a level of 83 mg/kg to liver sausage did not improve the protection (Table 8). This is in accordance to earlier findings found by Tompkin *et al.* (1978c) and ascribed to the inhibitory effect of the high iron content of this product.

Table 8. Protection from proteolytic *C. botulinum* of German-type canned sausage mixtures heated to Fo* = 0.34 and stored for 90 days (Lücke and Hechelmann, 1986)

Product	pH	a_W	Storage temp. (°C)	Protection** without and with $NaNO_2$	
				0 mg/kg	83 mg/kg
Brühwurst	6.0	0.982	21	4.0	6.9
Brühwurst	6.15	0.972	21	6.1	> 6.8
Brühwurst	6.15	0.972	30	5.1	6.6
Brühwurst	5.60	0.977	21	Not determined	7.0
Liver sausage	6.30	0.980	21	2.3	2.5
Liver sausage	6.20	0.970	21	3.6	3.5
Liver sausage	6.15	0.969	21	6.3	> 6.3
Liver sausage	6.15	0.969	30	3.8	5.0

*F_0= 0.34 means a heat process equivalent to 0.34 min at 121°C (e.g. 3.4 min at 111°C or 34 min at 101°C)

** Protection is defined as log (1/P) where P is the probability that a given spore survives the heat treatment and produces toxin in the product)

In Table 9, the protective action of nitrite in canned luncheon meat is illustrated using input concentrations of nitrite up to 150 mg/kg of product. In canned luncheon meat, protection was identical to that observed in Brühwurst. However, increasing the

[6] Protection is defined as the log (1/P) with P is the probability for a given spore to survive heat treatment and to produce toxin in the product.

concentration of nitrite from 75 – 83 mg/kg to 150 mg/kg did not further increase the protection.

Table 9. Protection of canned luncheon meat from proteolytic *C. botulinum* stored at 21°C. (Lücke and Roberts, 1993)

Fo*	a_W	Protection** without and with $NaNO_2$		
		0 mg/kg	75 - 83mg/kg	150 mg/kg
0.34	0.982	4.0	6.9	n.d.
0.64	0.973	n.d.	7.6	7.6
0.64	0.969	n.d.	7.5	7.8
0.64	0.966	n.d.	7.6	7.8

*F_0= 0.34 means a heat process equivalent to 0.34 min at 121°C (e.g. 3.4 min at 111°C or 34 min at 101°C)

** Protection is defined as log (1/P) where P is the probability that a given spore survives the heat treatment and produces toxin in the product

n.d. = not determined

From the above examples, and from other published information, it is clear that 50-100 mg/kg input sodium nitrite is adequate to protect many cooked cured meats against growth and toxin formation by *C. botulinum*. There is, however, a wide range of cured meats that are not cooked, produced under a wide range of circumstances and with varying levels of hygiene, in European countries from the Mediterranean to the Arctic Circle. In some of those circumstances an input level of sodium nitrite of 150 mg/kg is considered necessary to inhibit the growth of *C. botulinum*. In some of those uncooked products, relatively large pieces of meat are cured, and a nitrite input level of 150 mg/kg is necessary to ensure that all parts of the meat contain some nitrite after it diffuses into the bulk of the meat. As input levels are reduced, there is an increasing chance that parts of the meat will receive no nitrite, and those parts would present an increased risk of microbial growth, including growth of *C. botulinum*.

3. RESIDUAL LEVELS OF NITRITE IN CURED MEAT PRODUCTS

3.1. Factors affecting the residual levels.

The effects of several factors on the longevity of nitrite in a pork slurry system were described by Gibson et al. (1984). The conditions studied are listed at paragraph 2.2 above.
Results are summarised in Table 10, where "time to zero residual nitrite", was literally the time to < 10 mg/kg product.

Based on those results and additional data, the authors drew the following conclusions:

- the rate of nitrite loss was independent of the initial level added and the residual level was not clearly related to the level added;

- heat treatment had a significant effect on the residual level of nitrite, and the high heat treatment prolonged the time nitrite was detectable (see also Nordin, 1969);

- the rate of decrease of nitrite was significantly greater when ascorbate was present, and the decrease was even more pronounced in combination with high heat treatment;

- nitrite concentration fell more rapidly with increasing storage temperature.

The results indicated that monitoring of residual levels of nitrite in the final product is of limited value. The main reason is that the rate of loss of nitrite in a product is dependent on a number of factors including the heat process used, the pH of the product, the storage temperature and the addition of ascorbic acid or other reducing agents. Consequently the detection of low levels of nitrite will give no clue whether a product was recently manufactured with an initial low level of nitrite or was a product which had been stored for several months at a low temperature with an initial modest level of nitrite, or whether it was a product which contained in addition ascorbate. Hence, from a regulatory point of view, monitoring residual nitrite is of limited value.

Taken in conjunction with the tests for botulinum toxin that were made on every combination of factors tested, the presence of residual nitrite did not guarantee that the product would prevent growth of *C. botulinum*. Conversely, the absence of nitrite did not indicate that the product would support the growth of *C. botulinum*. The products that prevented growth of *C. botulinum* for the longest time at any storage temperature tested were those containing added ascorbate (or iso-ascorbate), which caused nitrite levels to decline rapidly and often contained no residual nitrite.

Many other studies have demonstrated that the added amount of nitrite is reduced rapidly in meat products. Hill et al. (1973) demonstrated that in frankfurters and sausages only 20 – 25% of the added nitrite is detectable in the final product after processing and storage for a week at 2-5°C. In a Danish study, Gry *et al*, (1983) reported that, in most cases, the reduction of nitrite levels is less than 50% in the first days after production, and may be reduced to less than 10% of the original concentration within some weeks of storage.

Table 10. Approximate number of days for residual nitrite to fall below <10 mg/kg in any of five replicates of a pork slurry system, pH 5.5-6.3. The NaCl levels (2.5, 3.5 and 4.5%) are combined in the table. (Based on data of Gibson et al., 1984).

Nitrite added (mg/kg)	Heat treatment					
	Unheated		Low heat 80°C/7 min		High heat 80°C/7 min + 70 °C/1 hour	
	Incubation temperature					
	15°C	35 °C	15 °C	35 °C	15 °C	35 °C
A) No addition						
100	5*	3	12	3	63	5
200	10	5	12	5	68	5
300	21	5	21	5	>168	21
B) as in A + ascorbate (1000 mg/kg)						
100	5	3	9	3	10	9
200	5	3	9	3	9	5
300	5	5	21	5	48	12
C) As A + ascorbate (1000 mg/kg) + polyphosphate 0.3 % (w/v)						
100	5	3	10	3	21	5
200	10	3	21	3	21	3
300	5	5	5	5	12	12

*Time in days

3.2. Surveillance data on residual levels of nitrites

It is generally accepted that monitoring for residual nitrite is of limited value unless the history of the product is known, and the residual levels do not indicate the "safety" of the product with respect to possible growth of *C. botulinum*. Nevertheless, considerable effort has been devoted to monitoring residual levels of nitrite in a range of meat products in North America and in most European countries.

Since the 1960s, the meat industry has changed and modified technologies of cured meat production, reduced the amounts of nitrite at input, and, often, incorporated ascorbate

into many products. These two activities have resulted in much reduced levels of residual nitrite.

Data on residual levels of nitrite in cured meats have been obtained from several EU Member States at the request of the DG Health and Consumer Protection to the national representatives in the Standing Committee on the Food Chain and Animal Health and to the Liaison Centre for the Meat Processing industry (CLITRAVI) to provide data on the technological need and real amounts of nitrites and nitrates in meat products in their country (SANCO A-12825). CLITRAVI considered that, the issue of lowering nitrite levels in meat products should be looked at in the framework of the CODEX ALIMENTARIUS, which lays down maximum ingoing nitrite levels which are even higher than the EU-levels.

In surveys of residual levels of nitrite in a wide range of cured meat products in France, Belgium, Ireland, Germany, Spain and the UK and also from Canada and the USA, very occasional samples contained levels of nitrite close to the permitted maximum residual amounts. The vast majority of samples complied with the current regulation. Such monitoring identifies bad manufacturing practices and excessive reliance on high levels of nitrite. However, it would not detect bad practice and use of high levels of nitrite if ascorbate is included in the formulation. Hence control of nitrite at input is more effective.

United Kingdom

In a survey on residual nitrite levels in processed meats, carried out 1997 on behalf of the Ministry of Agriculture, Fisheries and Food (MAFF), 200 samples were tested. The quantities of residual $NaNO_2$ ranged from < 0.2 mg/kg to 123 mg/kg for bacon and <0.2 to 170 mg/kg for other types the average concentration was found to be 24.4 mg/kg. The nitrite level of imported products was comparable to that of products produced in the UK.

France

In a survey of cured meat products in France in 1995 and 2002, 3,112 samples of raw dried, cooked and raw to be cooked, cured meat products were tested. In 1995, the quantities of residual $NaNO_2$ in 59% of samples of raw dried cured meat products ranged from 0 to 9 mg/kg, while in 2002 74% of samples of raw dried cured meat products ranged from 0 to 9 mg/kg. In the case of cooked cured meat products the figures were 56% in 1995 and 78% in 2002, showing the reduction on residual levels of cured meat products.

Belgium

Data on residual levels of nitrite in cured meat products have been obtained from Belgium at the request of SANCO for surveys carried out in 2002 and 2003. Data from 75 samples in 2002 showed that the levels of residual $NaNO_2$ in 76% of the samples were below 20 mg/kg. In 2003 after analyzing 24 samples the percentage was 71%.

Ireland

Data on residual levels of nitrite in cured meat products have been obtained from Ireland for surveys carried out in 2001 and 2002 with 147 samples of bacon and 386 samples of other cured meat products. In bacon, the levels of residual $NaNO_2$ were 0-20 mg/kg in 36% of the samples, 20–29 mg/kg in 20% of the samples, 30-39 mg/kg in 12% of the samples and 40-49 mg/kg in 7% of the samples.

Germany

Data on residual levels of nitrite in cured meat products have been obtained from Germany for surveys carried out in 2001 and 2002 with 116 samples. The levels of residual $NaNO_2$ in 85% of samples were below 20 mg/kg.

Canada

The Health Protection Brand of Health Canada published data on residual nitrite levels in Canadian cured meat products over the period 1972 -1996. The highest levels were detected in uncooked cured meats (pastrami, smoked beef, spiced beef), varying from 11-275 mg/kg of product. Over the period 1972 to 1996 there appeared to be a noticeable decline in the incidence of samples containing levels of residual nitrite higher than 100 mg/kg. However, average levels have decreased only slightly over the period of testing (Sen and Baddou, 1997).

At the time of the survey the maximum permissible input level under the Canadian Food and Drug Regulations was >200 mg/kg. The authors suggested that if the residual level exceeds 100 mg/kg in all probability the legal quantity at input has been exceeded.

USA

White (1975) calculated that cured meat manufactured in the United States contained on average 52.5 mg/kg of residual nitrite. Data used included nitrite values of 0 to 272 mg/kg. Cassens (1997) sampled bacon, bologna, sliced ham, and wieners at retail and found an average residual nitrite level of 10 mg/kg while the residual ascorbate was 200 mg/kg. He attributed these low levels to changing technologies, decreased levels of ingoing nitrite, and the use of ascorbate over the last 20 years.

3.3. Legal levels of nitrites used to cure meat products and suggestions from Members States and industry associations

United Kingdom

The current permitted levels of nitrite (and nitrate) are contained in Council Directive 95/2/EC, written into UK legislation in The Miscellaneous Food Additives Regulations 1995 (Statutory Instruments (SI) 1995/3187 as amended).

Germany

The current permitted limits for the use of nitrite and nitrate in meat products are compiled in the German Zusatzstoff-Zulassungsverordnung following the Council Directive 95/2/EC.

The German government suggests, according to a letter to the Commission dated 30. July 2003 (SANCO A-12825), the use of nitrite only as mixture with sodium chloride containing 0.4 to 0.5 % nitrite (as sodium nitrite), as was permitted in Germany before Directive 95/2/EC entered into force. In addition, the amount added to meat products should be limited and should not be higher than the quantities reported in the SCF Opinion of 19 October 1990 (section 3.1.1). Accordingly, 50 – 100 mg added nitrite (as sodium nitrite) per kg meat products may suffice for many purposes but some products may require higher concentrations up to 150 mg/kg.

According to the German Federal Centre for Meat Research (Bundesanstalt für Fleischforschung, Kulmbach), under certain circumstances such as strict conditions of hygiene, limited storage temperature and duration, the addition of nitrite to meat products is not necessarily required for preservation (letter of the German government to Commission dated 30 July 2003). Furthermore, the German government calls the use of nitrate generally into question (letter to Commission dated 30 July 2003).

Spain

The current permitted limits for the use of nitrite and nitrate in meat products are compiled in R.D. 142/2002 following the Council Directive 95/2/EC. The Confederation of Spanish Association of Meat Industries (Confecarne) answered a request of the Spanish Food Safety Agency in request to the letter from Commission for real levels of nitrates and nitrites. Confecarne considered that the amount of ingoing nitrite should not be reduced below 150 ppm for safety reasons. However, Confecarne considered that reduction of nitrate and nitrite levels in heat-treated meat products were possible, as follows: residual levels of nitrite could be reduced from 100 to 75 ppm and residual levels of nitrates from 250 ppm to 100 ppm. Indicative ingoing amount of nitrates could also be reduced from 300 ppm to 150 ppm.

Most of the added nitrite contributes to microbiological safety but a small part also contributes to other chemical activities like cured meat flavour, colour and antioxidative stability. The use of nitrate appears necessary in the case of particular traditional meat products like dry-cured ham. This is an entire piece of pork, and curing salt is rubbed on the outer surface and left for several weeks to allow diffusion through the entire piece (Toldrá, 2002). This is a very slow process that may take up to 2 months under cold storage, where the progressive diffusion of nitrate constitutes a reservoir of nitrite. In this way, nitrite effectively reaches the centre of the ham, especially critical locations like the joints, where nitrite protection is required before further maturation, consisting of increase of temperature for ripening and drying the product.

Italy

Italy reported that the limits on the use of nitrites and nitrates laid down by Directive 95/2/EEC are complied with. The Regulation concerns only maximal limits. Food producers are permitted to use lower levels of nitrates and nitrites in their production process or not to use these substances at all.

Denmark

The permitted levels in Denmark were substantial lower than those authorised by the Council Directive 95/2/EC (Table 15). Furthermore, the Danish legislation prescribed maximum amounts to be added; not indicative. The residual amounts in the EU column of Table 15 are estimates based on permissible added amounts and practical experiences concerning the disappearance of nitrite during storage.

Table 15. The Danish limits of added amounts of nitrite and nitrate as applied before introducing the EU legislation, and compared to those of Directive 95/2/EEC.

Product	DK	EU
Nitrites	Max. ingoing amount of nitrites in mg/kg	Indicative ingoing amount of nitrites in mg/Kg (Max, residual amounts in mg/kg)
Non heat treated meat products (whole pieces or cuts). In general:	60	150 (50)
Cured bacon, in general	60	Not limited (175)
Bacon of Wiltshire type (including cuts)	150	Not limited (175)
"Spegeskinker" (type of special cured hams)	150	150 (50)
Heat treated meat products (whole pieces or cuts), in general:	60	150 (50)
"Rullepølser" ("Rolled" sausages)	100	150 (50)
"Halv- og helkonserverede produkter" (Semi-preserved and full preserved meat products) and	150	150 (50)
Bacon of Wiltshire type (including cuts)	150	Not limited (175)
Non heat treated meat products of comminuted meat, in general:	60	150 (50)
Fermented "spegepølser" (salami-type sausages)	100	150 (50)
"Halv- og helkonserverede produkter" (Semi-preserved and full preserved meat products)	150	150 (50)
Heat treated products of comminuted meat, in general:	60	150 (50)
"Kødboller" og "leverpostej" (Danish meat balls and liver paté)	0	150 (50)
"Halv- og helkonserverede produkter" (Semi-preserved and fully preserved meat products)	150	150 (50)
Nitrates		
Cured meat products, in general	0	300 (250)
Bacon of Wiltshire type (including cuts) and "spegeskinker" (type of special cured hams)	300	300 (250)

4. CONCLUSION - ANSWERING TO THE TERMS OF REFERENCE

1) What is the correlation between in-going and residual amounts of nitrites and nitrates?

1A Nitrites

From the data available there is no simple and direct relationship between the in-going and the residual amounts of nitrite. The fate of nitrite added to meat is influenced by several factors including the pH of the meat product, the storage temperature, any heat treatment, and the presence of reducing substances e.g. ascorbate/isoascorbate.

1B Nitrates

It is also difficult to predict the fate of nitrate added to meat products. In some products it remains at essentially the concentration added, and then decays slowly. In some traditional products, nitrate acts as a reservoir of nitrite, the rate of reduction being dependent on the nature of the natural microbial flora. Occasionally nitrate can be detected in meat products to which only nitrite had been added, as the result of microbial activity.

2) What is the effect of the in-going and residual amount of nitrites and nitrates on the microbiological safety of meat products, in particular related to Clostridium botulinum*?*

2A Nitrites

Nitrite contributes to microbiological safety and also to the flavour, colour, and anti-oxidative stability of cured meat products. Most of added nitrite contributes to microbiological safety but a small part contributes to the other chemical activities.

The inhibitory effect of in-going nitrite is concentration- and pH-dependent, and the inhibitory effect is greater at acid pH values.

There is no convincing evidence that the residual amount of nitrite contributes to the microbiological safety of meat products. For example, in meat products containing ascorbate (or isoascorbate / erythorbate) the residual nitrite content is very low and sometimes below the level of detection, yet growth of *C. botulinum* is prevented.

The protective effect of nitrite is reduced in products with high iron content such as those containing liver or blood.

2 B Nitrates

In most meat products nitrate provides no direct protection against growth of *C. botulinum*. However, the use of nitrate as a reservoir of nitrite appears necessary in particular traditional dry cured meat products e.g. dry-ripened fermented sausages of high pH and dry cured ham. Such products have a good record of safety with respect to *C. botulinum*.

3) Which is the lowest level of in-going or residual amount of nitrite and nitrates which would still have a protective effect in meat products against microbiological risks, in particular related to Clostridium botulinum*?*

3A Nitrites

Several factors contribute to the safety of meat products including: cooking process, salt / brine concentration (a_w), storage time and temperature, pH and concentration of nitrite added. Hence, the lowest level of nitrite to have a protective effect against microbiological risks, such as *C. botulinum*, will be different in different products, depending on other factors including any heat treatment applied, the pH and the water activity /salt concentration.

Cured meat products with nitrite have an excellent record of safety with respect to *C. botulinum*. All laboratory tests indicate that sodium nitrite increases protection against *C. botulinum*.

Research in the 1960s and 1970s failed to identify an alternative to nitrite for cured meat products (NAS, 1982; Widdus and Busta, 1982).

The Panel is of the opinion that the ingoing amount of nitrite, rather than the residual amount, contributes to the inhibitory activity against *C. botulinum*. Therefore, control of nitrite in cured meat products should be via the input levels rather than the residual amounts.

The Panel agrees with the view of the Scientific Committee on Food (SCF) (expressed in section 3.1.1 of the Opinion of 19 October 1990) that 50 – 100 mg added nitrite (as sodium nitrite) per kg of meat products may suffice for many products. In other products, especially those with a low salt content and having a prolonged shelf-life, addition of between 50-150 mg/kg nitrite is necessary to inhibit the growth of *C. botulinum*.

The Panel agrees with the SCF which recommended in its Opinion of October 1990 that the use of nitrite should only be permitted as a mixture with salt (sodium chloride) to limit the amount of nitrite that can be added and to prevent accidental poisoning through the addition of excessive quantities of nitrite to food. The practicability of using salt containing 0.4 to 0.5% nitrite (as sodium nitrite) has been demonstrated over many years although using salt and nitrite separately has also been shown to be practicable in some countries with no accidental additions of high levels of nitrite over many years.

The Panel is of the opinion that the current "indicative ingoing amount" of potassium nitrite (E249) and sodium nitrite (E250) should be "maximum ingoing amount".

3B Nitrates

Nitrate per se has no protective activity against *C. botulinum*, but in some products is reduced to nitrite, which is active against *C. botulinum*.

The Panel is of the opinion that the current "indicative ingoing amount" of sodium nitrate (E251) and potassium nitrate (E252) should be "maximum ingoing amount".

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ACKNOWLEDGEMENT

The Scientific Panel on Biological Hazards wishes to acknowledge the contribution of the working group that prepared the draft opinion: S. Notermans (chair), T.A. Roberts (rapporteur), G.J. Nychas (members of the BIOHAZ Panel) and F. Toldrá (member of the AFC panel) and R. Gürtler (member of the WG on Food Additives from the AFC Panel).