ATTACHMENT 3

APPLICATION FOR ASSESSMENT OF THE FOOD ADDITIVE CARBON MONOXIDE (CO) PRIOR TO ITS AUTHORIZATION

(This application is based on the document "Presentation of an application for assessment of a food additive prior to its authorisation", Office for Official Publications of the European Communities, Luxembourg, 1989, ISBN 92-826-0135-8).

PART I. ADMINISTRATIVE DATA

I.1. **Applicants:** Altogether, the two applicants represent the total meat industry in Norway

Applicant no. 1:

The name of the applicant:

Norsk Kjøttsamvirke (Norwegian Meat Cooperative)

Address:

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Other means of communication:

Telephone: +47 22 09 21 00
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Applicant no. 2:

The name of the applicant:

Kjøttbransjens Landsforbund (The Norwegian Independent Meat Association) – represents the private meat industry in Norway

Address:

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P.O.Box 6279 Etterstad
0603 Oslo, Norway

Other means of communication:

Telephone: +47 23 24 44 70, Fax: +47 23 24 44 80

I.2. The name of the manufacturer(s) of the substance:

RIVOIRA S.P.A., Stabilimento Chivasso gas, Via cardinal Massaia 75L, I-10147 Torino, Italy

I.3. The name of the person responsible of the dossier:

Research director Truls Nesbakken, Norwegian Meat Research Centre, P. O. Box 396 Økern, 0513 Oslo, Norway. Telephone +47 22 09 23 99, Mobile phone +47 91 87 81 46, Fax +47 22 22 00 16, e-mail: truls.nesbakken@fagkjott.no

I.4. The table of contents of dossier

This dossier is sent through the Norwegian Food Control Authority (Statens næringsmiddeltilsyn). Together with this document follow as enclosures:

1) Nissen, H., Alvseike, O., Bredholt, S., Holck, A. and Nesbakken, T. (submitted) Packaging of ground beef in an atmosphere with high carbon dioxide and low carbon monoxide restrains growth of *Yersinia enterocolitica, Listeria monocytogenes* and *Escherichia coli* O157:H7. Int. J. Food Microbiol. As long as this work is not published, please handle this information with care.

2) Nissen, H., Alvseike, O., Bredholt, S., Holck, A. and Nesbakken, T. (1999) Packaging of ground beef in an atmosphere with low carbon monoxide and high carbon dioxide restrains growth of *Escherichia coli* O157:H7, *Listeria monocytogenes, Yersinia enterocolitica* and *Salmonella diarizonae*. In: Tuijtelaars, A.C.J., Samson, R.A., Rombouts, F.M., Notermans, S., (Eds.), Food Microbiology and food safety into the next millenium. Proceedings of the Seventeenth International Conference of the International Committee on Food Microbiology and Hygiene (ICFMH), 13-17 September 1999, Veldhoven, The Netherlands, pp. 285-286.

3) Solheim, R. (1996) Consumer purchase probability of beef and pork packaged in different atmospheres. Report, Matforsk, 10 pp.

4) Sørheim, O. (1996) Discoloration of meat as an indicator of leakages in packages containing a CO gas mixture. Report, Matforsk, 5 pp.

5) Sørheim, O., Aune, T. and Nesbakken, T. (1997a) Technological, hygienic and toxicological aspects of carbon monoxide used in modified-atmosphere packaging of meat. Trends in Food Sci. Technol. 8, 307 - 312

6) Sørheim, O., Nissen, H., and Nesbakken, T. (1999) The storage life of beef and pork packaged in an atmosphere with low carbon monoxide and high carbon dioxide. Meat Sci. 52, 157 - 164

7) Letter from the director of Swedish Meats (which is the organisation of the Swedish meat cooperative) supporting the Norwegian meat industry's application to the EU Commission

8) Letter from the director of Swedish Meat Trade Association (which is the organisation of the private meat industry in Sweden) supporting the Norwegian meat industry's application to the EU Commission

9) Letter from the director of the Danish Pig Producers and Slaughterhouses, Copenhagen, Denmark supporting the Norwegian meat industry's application to the EU Commission

10) Letter from the Spanish Meat Industry's Association supporting the Norwegian meat industry's application to the EU Commission

11) Letter from the Finnish Meat Research Institute supporting the Norwegian meat industry's application to the EU Commission

PART II. TECHNICAL DATA

II.1. Name of the substance

- names in the IUPAC nomenclature: carbon monoxide
- other names (usual name/trade name/synonyms: carbon oxide, carbon monoxide)
- abbreviations: CO
- CAS number (if this has been attributed): 630 – 08 – 0

II.2. Specification of the substance

- composition (%, m/v, mg/kg), e.g. in the case of heterogeneous products): 100%
- empirical and structural formula: CO
- molecular weight: 28.010 g/mole
- degree of purity (%): higher than 99.3%
- nature of known impurities/percentage of significant and main impurities: (< 0.7%):

Impurities:	Concentration:
Oxygen + Argon	< 2500 vpm
Water	< 20 vpm
THC (Total hydrocarbons)	< 500 vpm
Hydrogen + Nitrogen	< 3500 vpm
Carbon dioxide	< 500 vpm

- **physical form (liquid, powder, etc.):** gas

- **solubility (e.g. aqueous, organic solvents, lipid):**

solubility in water, 0°C, a CO partial pressure of 101.325 kPa = 3.537 $cm^3/100\ cm^3$. Solubility in organic solvents and lipid: not relevant – see Part II.6 Exposure

- **other data that the applicant believes may be useful to identify the substance (e.g. physico-chemical properties, analytical data on differences between batches):**

Thermodynamic properties of carbon monoxide as ideal gas at 25°C:
Heat capacity, c_p: 29.142 J/(mol * °K)
Entropy, S: 197.543 J/(mol * °K)
Enthalpy: 8.669 kJ/mol

- information on the microbiological characteristics, in particular on the possible presence of pathogens and bacterial or mycotoxins: not relevant

II.3. Manufacturing process

- **Information on the method of manufacture (i.e. the process by which the raw materials are converted to the finished product):**

The CO-gas is bought from RIVOIRA S.P.A., Torino, Italy (see I.2.). Hydro Rjukan Næringspark; P.O.Box 43-44, N-3661 Rjukan, Norway, makes the two CO-gas mixtures which are used in the Norwegian meat industry. They are called "Pakkemix NC1" and "DNC 29.7 – 0.3"

1) Pakkemix NC1 = 1.0% CO + 99% N_2

The production:

a) evacuation of an empty cylinder to under 10 mbar
b) flushing with N_2, quality 5.0
c) repeat the evacuation of an empty cylinder to under 1 mbar
d) manometric filling with CO, quality 2.3
e) manometric filling with N_2 quality 5.0
f) every tenth cylinder is analysed with gas chromatograph (GC) and thermoconductivity detector (TCD)

The pressure of the cylinder is 200 bar.

2) DNC 29.7 – 0.3 = 0.3% CO + 29.7% N_2 + 70% CO_2

The production:

a) evacuation of an empty cylinder to under 10 mbar
b) flushing with N_2, quality 5.0
c) repeat the evacuation of an empty cylinder to under 1 mbar
d) manometric filling with CO, quality 2.3
e) manometric filling with N_2, quality 5.0
f) manometric filling with CO_2, quality 3.0
g) every tenth cylinder is analysed with gas chromatograph (GC) and thermoconductivity detector (TCD)

The pressure of the cylinder is 50 bar..

II.4. Methods of analysis

- analytical methods to describe the substance, evaluate its purity and measure its physico-chemical and microbiological characteristics:

Oxygen + Argon, and Hydrogen + Nitrogen: Gas chromatograph with thermoconductivity detector (TCD) – min. detect. limits 10 vpm (RIVOIRA S.P.A., Stabilimento Chivasso gas, Via cardinal Massaia 75L, I-10147 Torino, Italy)

THC: Gas chromatograph with flame ionization detector (FID) – min. detect. limit 0.5 vpm (RIVOIRA S.P.A., Stabilimento Chivasso gas, Via cardinal Massaia 75L, I-10147 Torino, Italy)

Carbon dioxide: Gas chromatograph with helium ionization detector (HID) – min. detect. limit 0.5 vpm (RIVOIRA S.P.A., Stabilimento Chivasso gas, Via cardinal Massaia 75L, I-10147 Torino, Italy)

Water: Specific water analyzer – min. detect. limit 0.1 vpm (RIVOIRA S.P.A., Stabilimento Chivasso gas, Via cardinal Massaia 75L, I-10147 Torino, Italy)

- **analytical methods for the determination of the additive and its degradation products (where relevant), in the foodstuff of which the substance is to form part:**

The isotope, C^{14}, might be used for measuring CO before and after heat treatment (Watts et al., 1978). Spectrophotometry is used to measure carboxymyoglobin at 540 and 577 nm (native) or 425, 542 and 570 nm (denaturated) (El-Badawi et al., 1964; Cornforth, 1994).

II.5. Justification for the additive

- intended use and purpose:

Fresh red meat (mainly beef, pork and lamb, but also horse, goat, reindeer, game etc.) packaged in an atmosphere with 60 - 70% carbon dioxide (CO_2), 30 - 40% nitrogen (N_2) and < 0.5% carbon monoxide (CO) (high CO_2/low CO mixture).

Gas mixtures with low concentrations of CO and high concentrations of CO_2 provide a combination of a long microbiological shelf life and a stable bright red colour of meat (Sørheim et al., 1999).

- the quantity to be added to specific foods and the residues in food: < 0.5% CO.

- investigations on the efficacy of the substance for the intended effect at the level proposed:

The main function of low levels of CO in modified atmospheres (MAs) is to give a stable, cherry red colour of the meat through strong binding of CO to myoglobin and formation of carboxymyoglobin (El-Badawi, 1964). Although a substantial increase in the shelf life of meat can be obtained by using various MAs, it is often limited by discolouration due to oxidation of myoglobin to metmyoglobin. This discolouration can be prevented by including a small fraction of CO in the gas mixture. Carboxymyoglobin is more resistant to oxidation than oxymyoglobin, due to the stronger binding of CO to the iron-porphyrin site on the myoglobin molecule (Wolfe, 1980). CO in concentrations of 1 - 5% had the ability to increase metmyoglobin reduction, even in the presence of air (Lanier et al., 1978).

The high CO_2/low CO mixture and absence of O_2 provides a unique combination of a long microbiological shelf life (caused by the high CO_2 level) and a stable cherry red colour (caused by the low CO level). CO_2 inhibits growth of many microorganisms, but it has no effect *per se* on the colour of the meat (Renerre and Labadie, 1993). This gas is absorbed in meat and fat tissue at a ratio of approximately 1 litre gas per kg tissue (Gill, 1988). N_2 affects neither the microbiology nor the colour of the meat, but prevents collapse of the packages because it is not absorbed in the product. O_2 supports the growth of aerobic microorganisms, and removal of O_2 from the MA will therefore extend the microbiological shelf life. The shelf life of meat is considerably longer in the high CO_2/low CO mixture than in the commonly used atmosphere of high oxygen (O_2) with approximately 70% O_2 and 30% CO_2. Consumption of meat treated with the high CO_2/low CO mixture will result only in negligible levels of carboxyhaemoglobin in blood. It is highly improbable that CO from packaging of meat will present a toxic threat to the consumer (Sørheim et al., 1997a).

Shelf life in the high CO_2/low CO mixture in comparison with alternative packaging methods:

Ground beef, beef loin steaks and pork chops were packaged in MAs of 0.4% CO/ 60% CO_2/ 40% N_2 (high CO_2/low CO mixture) and 70% O_2/ 30% CO_2 (high O_2 mixture). In addition ground beef was packaged in clipped chub packs, beef loin steaks were vacuum packaged, and pork chops were packaged in an atmosphere of 60% CO_2/ 40% N_2 with each pack containing an

O_2 absorber. The packs were stored in the dark at 4°C or 8°C for up to 21 days. Meat in the high CO_2/low CO mixture had a stable bright red colour. The storage lives in this gas mixture at 4°C, as limited by off-odours, were 11, 14 and 21 days for ground beef, beef loin steaks and pork chops, respectively. The high O_2 mixture resulted in an initially bright red to red colour of the meat, but the colour was unstable and off-odours developed rapidly. The off-odours probably were caused by *Brochothrix thermosphacta*, which grew in all meat types, and in ground beef by pseudomonads also. Meat stored in chub packs, vacuum packs and 60% CO_2/ 40% N_2 with an O_2 absorber developed off-odours and microflora similar to those of meat in low CO/high CO_2 mixture with however less acceptable colours or appearances. These results show that a low CO/high CO_2 atmosphere is effective for preserving retail-ready meat (Sørheim et al., 1999).

Aspects of spoilage:

Consumers may evaluate the shelf life of packaged meat based on its colour. A possible negative aspect of using CO in modified atmosphere packaging (MAP) of retail meat is a concern that the consumer might misjudge the product, because the microbiological status may be masked by the stable cherry red carboxymyoglobin colour (Knopf, 1980). However, the consumer is able to detect spoilage by off-odour (Sørheim et al., 1999). This is in contrast to ready to eat products such as cooked, sliced vacuum or gas packaged meat, gas packaged vegetables and vacuum-packaged cheeses where the consumers often have to taste it before judging the product as unacceptable. As ready to eat products, they also represent a higher risk than fresh meat packed in the high CO_2/low CO mixture which is heat treated before consumption. In the current low concentrations, below 0.5%, CO *per se* seems to have no or only minor effects on bacteria and the shelf life of the meat. The combination of CO with high concentrations of CO_2, for example 60 - 70%, is necessary for microbiological control. MAP enables centralised packaging operations with quality control, but MAP alone is no guarantee for the shelf life of the product. Sufficient shelf life can only be obtained through a proper quality control of raw materials, production, packaging, chill chain and retail conditions (Sørheim et al., 1999).

Pathogens in the high CO_2/low CO mixture in comparison with alternative packaging methods:

Growth of the pathogens *Yersinia enterocolitica, Listeria monocytogenes, Escherichia coli* O157:H7 and strains of *Salmonella* was compared in ground beef packed in high CO_2/low CO mixture, high O_2 mixture and in chub packs. The ground beef was inoculated with rifampicin or nalidixic acid/streptomycin-resistant strains (final concentration 10^2-10^3 bacteria/g) and stored at 4°C and 10°C for up to 14 days. At 4°C the shelf life based on stable colour and reduced background flora was prolonged for the high CO_2/low CO mixture, compared to the two other packaging methods, but at 10°C the shelf life was < 8 days for all the packaging methods. Growth of *Y. enterocolitica* was nearly totally inhibited both at 4°C and 10°C in the high CO_2/low CO mixture, while the bacterial numbers in the samples packed in the high O_2 mixture increased from about $5x10^2$ bacteria/g at day 0 to about 10^4 at day 5 at 4°C and to 10^5 at 10°C. Growth in the chub packs was even higher. *L. monocytogenes* showed very little growth at 4°C in all treatments. At 10°C there was slow growth of *L. monocytogenes* from about $5x10^3$ bacteria/g to about 10^4 at day 5 in the high CO_2/low CO mixture, while the numbers in the high O_2 mixture and the chub packs were about 10 times higher. Growth of *E. coli* O157:H7 at 10°C in the ground beef was nearly totally inhibited in both the high CO_2/low CO mixture and the high O_2 mixture. Growth of *E. coli* O157:H7 in the chub packs was higher reaching 10^5 bacteria/g on day

5. The *Salmonella* strains (*S. Typhimurium, S. Dublin, S. Enteritidis* and *S. enterica* «61:k1, 5,(7))» in the ground meat stored at 10°C for 5 and 7 days, grew to a higher number in the high CO_2/low CO mixture than in the high O_2 mixture. The present study shows that the prolonged shelf life at 4°C did not increase growth of *Y. enterocolitica* and *L. monocytogenes* in ground beef stored in the high CO_2/low CO mixture, but the observed growth of strains of *Salmonella* in this mixture and in chub packs at the abuse temperature of 10°C does emphasise the importance of temperature control during storage (Nissen et al. 1999; Nissen et al., submitted).

Consumer purchase probability:

Ground beef, beef loin steaks and pork chops were packaged in high CO_2/low CO mixture and high O_2 mixture. In addition ground beef was packaged in clipped chub packs, beef loin steaks were vacuum packaged, and pork chops were packaged in an atmosphere of 60% CO_2/ 40% N_2 with each pack containing an O_2 absorber. The purchase probability data were collected by interviewing 126 consumers usually purchasing meat and meat products. The consumers visually compared the samples within each type of meat after 4 days storage at 4°C. The consumers preferred ground beef packaged in the high CO_2/low CO mixture or the high O_2 mixture compared to ground beef packaged in clipped chub packs. Purchase probability increased when pork chops were packaged in the high CO_2/low CO mixture. Pork chops in packs containing an O_2 absorber, were rated lowest in purchase probabilities. The purchase probability for beef loin steaks was similar when packaged in the high CO2/low CO mixture or the high O2 mixture, and these products were preferred compared to beef loin steaks packaged in vacuum (Solheim, 1996)

- documentation on the need for the additive:

Alternatives to the high CO_2/low CO mixture:

The most common MA for retail packaging of meat today contains O_2 at high concentrations combined with CO_2, approximately 70% O_2/ 30% CO_2. The shelf life of meat in high O_2 atmospheres in commercial practice, typically at temperatures of 6 - 8°C, is about 7 days, limited by both microbiological spoilage and discolouration. Meat stored in the high O_2 mixture is often spoiled by bacteria like *Brochothrix thermosphacta* and pseudomonads (Gill, 1996). In MAs with high concentrations of O_2, the meat normally maintains its bright red oxymyoglobin colour for 4 - 7 days before the colour starts deteriorating into grey/brown due to formation of metmyoglobin (Sørheim et al., 1999). This length of time is often not considered sufficient for displaying and selling the product to 4.5 mill. inhabitants all along the distance from Kristiansand in the south to Kirkenes in the north of Norway (about 2700 km) corresponding to the distance from Oslo to Rome (about 2600 km)!

Using high CO_2, MAs of either CO_2 alone or CO_2/N_2 with up to 70% CO_2 increases the microbiological shelf life of the meat compared to MAs of high O_2. The absence of O_2 combined with the presence of CO_2 retard the microbiological growth. Unfortunately, the colour of the meat in MAs of CO_2 is less satisfactory, either as purple deoxymyoglobin or as grey/brown metmyoglobin. The meat inevitably discolours when concentrations of O_2 are low. Tolerance levels for avoiding metmyoglobin formation are less than 0.1% O_2 for beef (Gill and McGinnis, 1995) and 0.5% O_2 for pork (Sørheim et al., 1997b). These low O_2 levels, particularly for beef, are difficult to achieve in most commercial packaging operations, because small fractions of air will be

incorporated in the MAs of the packages. MAs with high CO_2 concentrations seem to be useful for retail packaging when combined with low concentrations of CO for stabilisation of myoglobin and the meat colour.

Vacuum is a packaging method commonly used for bulk storage, transportation and export of meat. However, vacuum has not been a successful method for retail packaging of meat, because of the purple deoxymyoglobin colour of the meat and the visible exudate that occurs in the packages (Bruce, 1990; Gill, 1996). Meat packaged in vacuum can not be presented in the bright red oxymyoglobin state, which depends on the presence of high concentrations of O_2 (Gill, 1996; Taylor et al., 1990), or alternatively as cherry red carboxymyoglobin with CO included in the MA.

Hazard for workers:

One of the objections raised against CO as a component of a packaging gas is the potential hazard it might represent for the workers in the meat plants. Using pure CO for mixing in the plant would certainly be such a risk, however, CO is delivered as a premixture (DNC 29.7 – 0.3) or in a 1% mixture together with 99% N_2 (Pakkemix NC1), which is the practice of gas suppliers to the Norwegian meat industry. This way of supplying CO is recognised to be a very safe handling procedure by the health authorities. MAs with concentrations of 60 - 70% O_2 must be handled carefully, because they are explosive gas mixtures. Strict safety regulations apply to explosive gas mixtures, resulting in additional costs of equipment and packaging operations. The benefit of the CO mixture is that it carries no risk or handling costs due to risk of explosion.

Experience of the Norwegian meat industry:

Despite the long term knowledge of CO and its many positive properties as a component of MAs for meat, the CO mixture has not been adopted to any large extent by the global meat industry. In many countries, like the US and EU, CO is presently not permitted for use in MAP of meat (Cornforth, 1994; European Parliament and Council Directive, 1995). However, the Norwegian food control authority has derogated from the EU directive for a two years period. Accordingly, the Norwegian meat industry might use CO as a component of a packaging gas in concentrations up to 0.5% until October 1, 2000. The high CO_2/low CO mixture is the only MAP which provides a shelf life sufficient for displaying and selling fresh retail meat products in all parts of Norway. The Norwegian meat industry started to use the high CO_2/low CO mixture in packaging of fresh retail meat products in the mid-eighties. The market share of retail meat packaged in the high CO_2/low CO mixture in Norway is currently estimated at 50 - 60% (ground beef as high as 85%). The Norwegian food control authority has not registered outbreaks or a higher frequency of sporadic cases of food borne diseases linked to such products (The Scientific Committee, under The Norwegian Food Control Authority, 19.4.99).

Support from the European meat industry:

The meat industry in Sweden has also discovered the benefits and advantages of the high CO_2 /low CO mixture in packaging of fresh meat. Both Swedish Meats (which is the organisation of the Swedish meat cooperative) and the Swedish Meat Trade Association (which is the organisation of the private meat industry in Sweden) support the Norwegian meat industry's

application to the EU Commission (letters enclosed). Also, the Danish Pig Producers and Slaughterhouses, the Spanish Meat Industry and the Finnish Meat Research Institute support the application (letters enclosed).

- **benefit for the consumer:**

The high CO_2/low CO mixture is the only MAP which provides a shelf life sufficient for displaying and selling fresh retail meat products in a large geographical area like Norway.

Food safety and traceability:

The high CO_2/low CO mixture enables centralised packaging operations with quality control with less risk for cross-contamination than in local butcher shops or by supermarket back-store operations. The Norwegian meat industry already produces pork products traceable in integrated systems back to the farm and beef products traceable to the individual animal.

The ability of *Y. enterocolitica* to multiply at low temperature is of considerable concern to food producers, particularly in countries like Australia, Canada, Denmark, Germany, New Zealand, Norway and Sweden where *Y. enterocolitica* has surpassed *Shigella* and now rivals *Salmonella* and *Campylobacter* as a cause of acute bacterial gastroenteritis (Nesbakken, 2000). The growth of *Y. enterocolitica* was totally inhibited in ground beef packed in the high CO_2/low CO mixture both at 4°C and 10°C while it grew fairly well both in the high O_2 mixture and in the chub packs.

At the abusive storage temperature of 10°C *E. coli* O157:H7 in the chub packs grew about as fast as the background flora. However, growth was nearly totally inhibited in the high CO_2/low CO mixture and in the high O_2 mixture (Nissen et al., 1999; Nissen et al., submitted).

Quality:

Centralised pre-packaging of retail meat in the meat industry is cost-effective compared to on-site packaging in food stores. Self-service food stores and supermarkets often require to be supplied with pre-packaged meat. The long shelf life of meat packaged in the high CO_2/low CO mixture provides a possibility of a wider selection of fresh meat on display in the stores. If the Norwegian meat industry looses the possibility to use this mixture, food stores in rural and remote areas will have to be supplied by frozen meat, which has a low acceptability of the consumer.

The high CO_2/low CO mixture provides extended freshness: fresh meat will last for many days (often more than a week) in the consumer's home refrigerator, and the consumer might shop fresh meat once a week, and fresh meat is available 24 hrs a day 7 days a week in hypermarkets, supermarkets, discount stores, service stores; and the consumer might get quality premium brand fresh meat in her/his local discount store. The consumer will find a wider variety of fresh meat products than otherwise possible.

The consumers seem to prefer fresh meat products packaged in the high CO_2/low CO mixture or the high O_2 mixture compared to other packaging methods (Solheim 1996).

Leakages in packages containing high CO_2/low CO mixture might be detected by the consumer. The discoloration might be an indicator of leakages for ground beef packed in the high CO_2/low CO mixture (Sørheim, 1996).

Prices:

Industrialised handling with centralised packaging in MAP means lower consumer prices. Waste due to "sell by date" in the distribution chain in Norway (high CO_2/low CO mixture) is less than 1%, as compared to 2 – 3% in countries using the high O_2 mixture, according to interviews with operators/supermarket chains in UK, The Netherlands and Spain (Dag Hallan, Norwegian Meat Cooperative, personal communication).

II.6. Exposure

Carbon monoxide (CO) is a colourless, odourless and tasteless gas. It is produced by incomplete combustion of carbon-containing organic material. The production of CO from natural processes is quite significant. Nevertheless, CO from antropogenic activities is far more important concerning human health, since this formation takes place in heavily polluted areas.

Natural background levels of CO are 0.01 - 0.9 mg/m^3 (0.01 - 0.8 ppm). In urban areas, 8-h mean concentrations of CO are generally < 20 mg/m^3, but levels exceeding 60 mg/m^3 have been reported (WHO, 1979). Among tobacco smokers, CO from smoking is by far the dominating source of CO exposure (WHO, 1987).

According to WHO experts (WHO, 1979; WHO, 1987), the only way of exposure which is of relevance to human health, is via inhalation of CO gas. Upon absorption from the lungs into the blood, CO forms a strong coordination bond with the iron atom in haemoglobin forming carboxyhaemoglobin (HbCO). The affinity of haemoglobin for CO is roughly 240 times that of its affinity for oxygen. CO is absorbed through the lungs and the concentration of HbCO in the blood will depend on several factors, mainly the concentration of CO in inhalation air, the exposure time and the level of activity of the individual (pulmonary ventilation).

The Norwegian meat industry is using a gas mixture of 60 - 70% CO_2, 30 - 40% N_2 and 0.3 - 0.4% CO for the packaging of fresh retail meat of beef, pork and lamb. According to Watts et al. (1978) beef which is exposed to an atmosphere containing 1% CO for 3 days result in about 30% saturation of the meat myoglobin. When the meat was cooked (hotplate maintained at 195°C for up to 8 minutes), only 0,1 mg CO remained in the meat per kg resulting in a loss of CO about 85%.

Data are very scarce concerning comparison of CO exposure from air and consumption of CO-treated meat. According to Sørheim et al. (1997a) consumption of 250 g CO-treated meat (after cooking) yield a theoretical intake of maximum 0.025 mg CO, compared with inhalation of 15 mg CO per hour at the acceptance level suggested by Norwegian authorities (giving 1.5% HbCO, including endogenous formation). Even though the estimates are crude, the calculations show without doubt that intake of CO from meat consumption is negligible. Furthermore, absorption of CO from the gastrointestinal tract will be very much lower (if it happens at all), compared with absorption via the lungs.

II.7. Reaction and fate in food

The main function of low levels of CO in MAs is to give a stable, cherry red colour of the meat through strong binding of CO to myoglobin and formation of carboxymyoglobin (El-Badawi, 1964). Although a substantial increase in the shelf life of meat can be obtained by using various MAs, it is often limited by discolouration due to oxidation of myoglobin to metmyoglobin. This discolouration can be prevented by including a small fraction of CO in the gas mixture. Carboxymyoglobin is more resistant to oxidation than oxymyoglobin, due to the stronger binding of CO to the iron-porphyrin site on the myoglobin molecule (Wolfe, 1980). CO in concentrations of 1 - 5% had the ability to increase metmyoglobin reduction, even in the presence of air (Lanier et al., 1978).

PART III. TOXICOLOGICAL DATA

III. 1 - 4.

Health effects of carbon monoxide has been evaluated by two WHO expert groups (WHO, 1979, WHO, 1987). The health effects are associated with the degree of HbCO formation. According to the aforementioned expert groups, the most sensitive individuals should be protected from CO exposures leading to a HbCO level exceeding 2.5%. In healthy adults, no adverse health effects are described at concentrations resulting in HbCO levels < 5%.

A small amount of CO is formed naturally in the human body, from breakdown of haemoproteins. This production leads to a HbCO concentration of about 0.5%. The average HbCO concentration in non-smokers is 1.2 - 1.5%, while the level in smokers usually is 3 - 4%.

The WHO experts (WHO, 1987) recommended a maximum HbCO level of 2.5 - 3% in order to protect the population at large, included sensitive individuals. In order to obtain this, they recommended maximum levels of CO in ambient air which will meet this requirement for different times of exposure:

Maximum levels of 100 mg/m^3 for < 15 min
Average levels < 60 mg/m^3 for 30 min
Average levels < 30 mg/m^3 for 1 hour
Average levels < 10 mg/m^3 for 8 hours

The European Union has not evaluated CO for use as a packaging gas for meat. However, in 1990 (European Commission, 1991), several other gases (carbon dioxide, oxygen, nitrogen, nitrous oxide, hydrogen and argon) were evaluated by the Scientific Committee for Food (SCF) for use as packaging gases and propellants. In this case it was considered unnecessary to adopt ADIs because of general knowledge of their safety in use, and the estimated insignificant intakes compared with exposure from other sources. Furthermore, in 1996, the SCF reviewed the safety of modified and controlled atmosphere packaging again, and placed particular emphasis on the importance of HACCP for the avoidance of microbiological risk in this context (European Commission, 1996). The SCF concluded that it does not see specific hazards for human health by

the use of controlled or MAs, but that a prerequisite is that the principles of HACCP are observed. A similar approach should also be feasible concerning CO used at very low concentrations in mixture with CO_2 and N_2.

Accordingly, the issue which should be solved concerning health effects of CO used in gas packaging, is the question of preventing the consumers from exposure to meat of unacceptable microbiological quality. Thus, two studies on shelf life, off-odour and colour (Sørheim et al., 1999) and pathogens (Nissen et al., 1999; Nissen et al., submitted) follow as enclosures. Summaries of the two studies are also given in "Part II.5. Justification for the additive - investigations on the efficacy of the substance for the intended effect at the level proposed".

III.5. Review of results and conclusions

As can be seen from the foregoing, exposure to CO via consumption of meat products treated with a MA containing < 0.5% CO represent a negligible source of CO, and will probably not contribute to any increase in the carboxyhaemoglobin level. From a toxicological point of view, packaging gas with < 0.5% CO presents no threat to human health (Sørheim et al. 1997a). This is in accordance with an assessment performed by members of The Scientific Committee for Food, under The Norwegian Food Control Authority (30.11.98).

PART IV. SUMMARY DOCUMENT

Gas mixtures with low concentrations of CO and high concentrations of CO_2 provide a combination of a long microbiological shelf life and a stable bright red colour of meat. Meat packaged in a MA with high O_2 can achieve an initial bright red colour, but the microbiological shelf life and the colour stability are considerably lower than those of the CO mixture. Using CO eliminates the need for having O_2 as a component of the MA. Other MAs and packaging methods, like high CO_2 with O_2 absorbers, chub packs and vacuum packs may give a microbiological shelf life similar to that of the high CO_2/low CO mixture, but with a less acceptable colour or appearance of the meat. Thus, there appears at present to be no fully satisfactory alternatives to the CO mixture used in packaging of retail-ready red meats in Norway (Sørheim et al., 1999).

In an investigation, growth of *E. coli* O157:H7 at 10°C in ground beef was nearly totally inhibited in the high CO_2/low CO mixture. The prolonged shelf life at 4°C did not increase growth of *L. monocytogenes* in ground beef stored in the high CO_2/low CO mixture. The growth of *Y. enterocolitica* was totally inhibited in ground beef packed in the high CO_2/low CO mixture both at 4°C and 10°C while it grew fairly well both in the high O_2 mixture and in the chub packs. However, the observed growth of strains of *Salmonella* both in the high CO_2/low CO mixture and in chub packs at the abuse temperature of 10°C does emphasise the importance of temperature control during storage (Nissen et al., 1999; Nissen et al., submitted).

From a toxicological point of view, packaging gas with < 0.5% CO presents no threat to human health (Sørheim et al. 1997a). The European Union has not evaluated CO for use as a packaging gas for meat. However, in 1990 (European Commission, 1991), several other gases (carbon dioxide, oxygen, nitrogen, nitrous oxide, hydrogen and argon) were evaluated by the Scientific

Committee for Food (SCF) for use as packaging gases and propellants. In this case it was considered unnecessary to adopt ADIs because of general knowledge of their safety in use, and the estimated insignificant intakes compared with exposure from other sources. A similar approach should also be feasible concerning CO used at very low concentrations in mixture with CO_2 and N_2.

The Norwegian meat industry started to use the high CO_2/low CO mixture in packaging of fresh retail meat products in the mid-eighties. The market share of retail meat packaged in the high CO_2/low CO mixture in Norway is currently estimated at 50 - 60% (ground beef as high as 85%). The Norwegian food control authority has not registered outbreaks or a higher frequency of sporadic cases of food borne diseases linked to such products (The Scientific Committee, under The Norwegian Food Control Authority, 19.4.99).

Conclusions:

Gas mixtures with low concentrations of CO, up to 0.5%, and high levels of CO_2, approximately 70%, have many advantages regarding shelf life, inhibition of pathogenic bacteria like *E. coli* O157 and *Y. enterocolitica*, colour stability, labour safety and costs. CO used as described in these concentrations, does not present any toxic threat to the consumer. Considering the benefits the Norwegian meat industry has experienced with the CO gas mixture over the past decade, this gas mixture should have a potential for a wider application in retail packaging of meat in the EU.

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