GR

ORIGINAL SUBMISSION

000003

ERIC F. GREENBERG
ATTORNEY AT LAW

ERIC F. GREENBERG
Of Counsel
Ungaretti & Harris

Contains Confidential Business Information

RECEIVED
4 2001
OFFICE OF
PREMARKET APPROVAL

August 29, 2001

Division of GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C St., SW
Washington, DC 20204

Re: NOTIFICATION OF CLAIM FOR GENERAL RECOGNITION OF SAFETY OF CARBON MONOXIDE IN A MODIFIED ATMOSPHERE SYSTEM FOR PACKAGING FRESH MEAT, submitted by Pactiv Corporation

To the FDA:

Enclosed is the NOTIFICATION OF CLAIM FOR GENERAL RECOGNITION OF SAFETY OF CARBON MONOXIDE IN A MODIFIED ATMOSPHERE SYSTEM FOR PACKAGING FRESH MEAT, submitted by Pactiv Corporation, 1900 West Field Court, Lake Forest, Illinois 60045, c/o the undersigned counsel, consisting of pages 000001.001 through 000250.

Please note that this submission contains Confidential Business Information that Pactiv Corporation desires not to be revealed to Freedom of Information Act requestors and other members of the public. In the first copy of the submission following this letter, the Confidential Business Information has been redacted. For ease of reference, a list indicating which pages contain redactions is attached.

Five complete copies of the submission are enclosed, including the one that has been redacted.

If you have any questions, please contact me at 312 977-4647.

Sincerely

Eric F. Greenberg
Enc.

000004

[redacted]

efgreenberg@uhlaw.com
3500 THREE FIRST NATIONAL PLAZA, CHICAGO, ILLINOIS 60602-4283
PHONE: 312-977-4647 · FAX: 312-977-4405

REDACTIONS

000003	000020	000056
000005	000026	000057
000006	000027	000160
000008	000028	000162
000009	000033	000166
000013	000038	000199
000016	000040	000222
000017	000041	000248
000018	000043	
000019	000054	

[redacted]

Notification of Claim for General Recognition of Safety of Carbon Monoxide in A Modified Atmosphere System for Packaging Fresh Meat

TABLE OF CONTENTS

Page

GRAS Notice 000003

I. **Claim of Exemption** 000004

a. **Name and address of the notifier**. 000004
b. **Common or unusual name of the notified Substance** 000004
c. **Conditions of use (foods, levels, purposes)**. 000005
Summary 000005
"Traditional" ActiveTech 000006
AT2001 000007
d. **Basis of GRAS determination: Scientific procedures** 000011

CO Safety 000011

Effects on Fresh Meat and Consumption 000015

Safety: Effects on carboxymyoglobin levels 000016

Safety: The Norwegian experience 000025

Safety: Exposure in environment 000026

Corroborative information about AT2001. 000029

e. **Statement of availability of information** 000038

II. **Identity of notified substance** 000038

III. **Self-limiting levels of use**. 000040

IV. **Basis of GRAS determination**. 000040

Conclusion 000043

000004.002

[redacted]

Page

Attachments:

Attachment 1	Authorization letter from Pactiv Corporation for representation by Eric F. Greenberg	**000050**
Attachment 2	Photographs of meats treated with ActiveTech and ActiveTech 2001	**000053**
Attachment 3	"Application for Assessment of the Food Additive Carbon Monoxide (CO) Prior to its Authorization", Norwegian Meat Cooperative; Norwegian Independent Meat Association (1999)	**000058**
Attachment 4	"Evaluation of Beef Steaks and Ground Beef in the Pactiv ActiveTech Packaging System: Effects of Carbon Monoxide in the Package Atmosphere", Hachmeister, K; Hunt, M.; Milliken, G; May 2001	**000157**
Attachment 5	Certificate of Conformance, Carbon monoxide, Haun Welding Supply, Inc., Syracuse, New York, May 8, 2001	**000190**
Attachment 6	"Standard Practice for Analysis of Reformed Gas by Gas Chromatography", ASTM D 1946-90 (Reapproved 2000)	**000192**
Attachment 7	Daren Cornforth, Ph.D., letter and curriculum vitae	**000198**
Attachment 8	Vasilios H. Frankos, Ph.D., letter and curriculum vitae	**000205**
Attachment 9	Melvin C. Hunt, Ph.D., letter and curriculum vitae	**000221**
Attachment 10	Oddvin Sorheim, Ph.D., letter and curriculum vitae	**000247**

000004.003

[redacted]

ERIC F. GREENBERG
ATTORNEY AT LAW

ERIC F. GREENBERG
Of Counsel
Ungaretti & Harris

Contains Confidential Business Information

August 29, 2001

Division of GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C St, SW
Washington, DC 20204

Re: NOTIFICATION OF CLAIM FOR GENERAL RECOGNITION OF SAFETY OF CARBON MONOXIDE IN A MODIFIED ATMOSPHERE SYSTEM FOR PACKAGING FRESH MEAT

To the FDA:

This letter and its attachments contains the notification, pursuant to the Federal Food, Drug and Cosmetic Act and FDA's regulations, by Pactiv Corporation, 1900 West Field Court, Lake Forest, Illinois 60045, c/o attorney Eric F. Greenberg, 3500 Three First National Plaza, Chicago, Illinois 60602[1], for the General Recognition of Safety of carbon monoxide ("CO") at a level of 0.4% in a modified atmosphere system for packaging fresh meat.

[1] Attachment 1 contains Pactiv's authorization of undersigned counsel, as well as a Summary regarding Pactiv Corporation.

efgreenberg@uhlaw.com
3500 THREE FIRST NATIONAL PLAZA, CHICAGO, ILLINOIS 60602-4283
PHONE: 312-977-4647 · FAX: 312-977-4405

As set forth more fully below and in the attachments to this document, Pactiv believes its intended use of CO is GRAS based on scientific procedures within the meaning of 21 U.S.C. Sec. 201(s) and FDA's implementing regulations in 21 CFR Sec. 170.30, and including FDA's proposed rule published on April 17, 1997 (62 FR 18937). FDA regulations provide that the scientific evidence available and reviewed for a GRAS determination is of the same quantity and quality as that required for a food additive approval, and that the scientific evidence of safety be generally known and accepted by qualified experts in the appropriate scientific and technical fields. 21 CFR Sec. 170.30(a).

I. Claim of Exemption

a. Name and address of the notifier.

Pactiv Corporation
1900 West Field Court
Lake Forest, Illinois 60045
c/o Eric F. Greenberg
Attorney at Law
3500 Three First National Plaza
Chicago, IL 60602

b. Common or usual name of the notified substance.

Carbon monoxide ("CO")

000005.001

[redacted]

c. Conditions of use (foods, levels, purposes).

When used as described in this Notice, CO meeting appropriate purity specifications is a processing aid in packaging of fresh cuts of muscle meat and ground meat, as a component of a gas mixture utilized in a specific modified atmosphere packaging system. 21 CFR Sec. 170.3(o)(24). A technology utilizing 0.4% CO within a modified atmosphere packaging system will maintain wholesomeness, permit greater flexibility in distribution, and reduce shrinkage, all within a system that results in traditional product display to consumers. All elements of the system, excluding the CO, are already in use in the United States as part of a modified atmosphere meat packaging system called ActiveTech™. Notifier refers to the new system incorporating CO as "AT2001".

Summary

ActiveTech™ is a system that is designed to permit more extended storage of meats, but, as explained below, has no effects on retail display time or characteristics as compared with other modified atmosphere technologies currently in use. It employs materials that are either approved additives used consistently with their approvals, or GRAS substances. AT2001 adapts that system for additional storage scenarios. AT2001 serves to reduce the time needed for enzymatic reduction after modified

atmosphere packaging, and allows consistent display color of whole muscle meats. AT2001's advantages are in the resulting flexibility and consistency during storage and distribution.

The GRAS use of CO described in this Notice involves use as a component of the flush gas mixture used in replacement of ambient air in the packaging for distribution of refrigerated fresh red meat. The meats are in all instances fresh, and are intended to be cooked prior to consumption.

"Traditional" ActiveTech™

The ActiveTech™ modified atmosphere system, in commercial use in the United States since 1998, is a modified atmosphere system for packaging fresh cuts of muscle meat, or portions of ground meat. AT2001 is a refinement of ActiveTech™, and differs from it only in the addition of 0.4% CO to the modified atmosphere.

In the "traditional" ActiveTech™ system, the meats are placed in polystyrene trays and covered with oxygen-permeable, flexible polyvinyl chloride ("PVC") overwraps. The wrapped trays of meat are then placed within an outer barrier bag from which ambient air is removed and replaced with a blend of 30% carbon dioxide (CO_2) and 70% nitrogen (N_2). An activated oxygen-absorbing sachet is also added within the outer bag.

This modified atmosphere maintains the packaged meat in an oxygen-free deoxymyoglobin state, with its distinctive purplish appearance that is generally considered undesirable by consumers. The traditional ActiveTech™ system relies on the rapid reduction of the oxygen content of the outer bag. Once the oxygen is removed, a "seasoning" phase begins during which enzymatic effects take place so that the meat will be able to "re-bloom" when once again in the presence of oxygen. As the residual oxygen in the package is consumed by the activated oxygen scavenger, red meat oxymyoglobin is first subject to rapid conversion to metmyoglobin (brown) at very low partial pressures of oxygen, e.g. 0.5% oxygen. This low partial pressure region of oxygen is necessarily passed through prior to ultimately reaching 0% in the package and the conversion to deoxymyoglobin (purple). This seasoning phase can take up to 5 days.

Before display to consumers at retail, the outer bag, and thus the modified atmosphere, is removed, and the traditionally wrapped product (in polystyrene foam tray with PVC overwrap) is permitted to "re-bloom" to its familiar appearance through creation of oxymyoglobin on the meat's surface.

AT2001

In the AT2001 modified atmosphere system, as with traditional ActiveTech™, fresh cuts of muscle meat, or portions of ground meat, are placed in polystyrene trays and covered with oxygen permeable flexible PVC overwraps. The wrapped trays are placed within the outer barrier bag, the air is removed and replaced with a blend of 0.4% CO, 30% carbon dioxide (CO_2) and the balance nitrogen (N_2). As with the traditional AT system, an activated oxygen-absorbing sachet is added within the outer bag to create and maintain an oxygen-free environment for the packaged meat during storage.

As noted, meat packaged in traditional ActiveTech™ undergoes a myoglobin pigment conversion from oxymyoglobin (red) to metmyoglobin (brown) to deoxymyoglobin (purple) in the oxygen free environment. The metmyoglobin formed generally will convert to deoxymyoglobin in the oxygen free storage environment in about 5 days, a period of time referred to as the "seasoning period". However, the meat's ability to convert all of the metmyoglobin formed to deoxymyoglobin during the seasoning period and then fully rebloom to oxymyoglobin upon re-exposure to normal, oxygen-rich atmosphere at retail, is a function of a multitude of unpredictable, uncontrollable factors in the meat such as age, muscle

location, and enzyme energy level. This is a key weakness of all current low oxygen packaging systems.

Meat packaged in the AT2001 atmosphere will instead convert from oxymyoglobin to carboxymyoglobin (red) in the package due to the inclusion of 0.4% CO in the modified atmosphere. This conversion occurs during the initial 24 hours as the free oxygen in the headspace is consumed. Thus, the CO effectively protects the myoglobin from converting to metmyoglobin as the oxygen in the package is removed. This feature is especially important for the most pigment sensitive meats such as those from the round. The meat will continue to maintain its red color while in storage until the package is opened for retail display, when the outer bag (and modified atmosphere) is removed. Since carboxymyoglobin and oxymyoglobin are essentially the same colors, no seasoning period is necessary. The meat can be opened for retail display the day following packaging.

Once in retail display, the meat's myoglobin will begin the rather slow, natural conversion to metmyoglobin (brown), akin to that seen with untreated meat, allowing for a conventional retail display life of 3 to 4 days, closer to consumers' expectations of color than results from use of high

oxygen packaging systems. Attachment 2 consists of photographs depicting the ActiveTech™ and AT2001 systems.

The AT2001 formulation will assure that the meat will have the familiar color during and following storage, eliminating the seasoning period, allowing for placement in retail display beginning at 1 day, and up to 30-40 days, after packing. For cuts of meat from the round, and other color sensitive cuts, the AT2001 will help them have a more uniform red color for retail display.

In AT2001 (as in traditional ActiveTech™), the trays and films utilized are made from familiar, FDA-approved polymers that are used in accordance with their existing approvals or GRAS status. The activated oxygen-absorbing sachet inserted into the outer bag to absorb oxygen does not contact food and is not expected to become a component of the food. Therefore, it is not a food additive under the definition in 21 USC Sec. 321(s). As an added assurance of safety, each of the sachet's components has some GRAS status or food-related approvals.

Thus, the AT2001 system adds a refinement to the existing ActiveTech™ system that will allow its utilization with whole and ground meat products that meet the processors' desire to get to market as soon as the day following processing.

d. Basis of GRAS determination: Scientific procedures

CO safety

Pactiv believes its proposed use of CO is GRAS based on scientific procedures within the meaning of 21 U.S.C. Sec. 201(s) and FDA's implementing regulations in 21 CFR Sec. 170.30 and including FDA's proposed rule published on April 17, 1997 (62 FR 18937).

CO is a colorless, odorless gas that is poisonous to humans if inhaled at much higher levels than are involved with the use that is the subject of this Notice. It is formed when carbon is not completely burned, for example, in the combustion of fuels.

It is well known that CO creates negative health effects at elevated levels because it out-competes oxygen for attachment to the hemoglobin molecule. The resulting carboxyhemoglobin levels in the blood are associated with severe health effects. In addition, the equilibrium rate for the exchange of carboxyhemoglobin for oxyhemoglobin is very slow, and the resulting level of carboxyhemoglobin is a function of the CO level in the respired air, the time of exposure and the level of activity of the individual. Typical atmospheric CO levels are <20 mg/m^3 as an 8 hour mean (higher in

urban and high traffic areas), and typical carboxyhemoglobin levels due to natural background CO range between 1.2 and 1.5%.

CO is recognized as a significant air pollutant at higher levels. Automobile exhausts, industrial processes and boilers and incinerators all contribute to air quantities of CO. According to the U.S. EPA Office of Air and Radiation:

> Carbon monoxide enters the blood stream and reduces oxygen delivery to the body's organs and tissues. The health threat from exposure to CO is most serious for those who suffer from cardiovascular disease. Healthy individuals are also affected, but only at higher levels of exposure. Exposure to elevated CO levels is associated with visual impairment, reduced work capacity, reduced manual dexterity, poor learning ability, and difficulty in performing complex tasks. EPA's health-based national air quality standard for CO is 9 parts per million (ppm) [10 mg/m^3] measured as an annual second-maximum 8-hour average concentration.

"Summary regarding carbon monoxide" as part of discussions of 6 principal pollutants, U.S. EPA Office of Air and Radiation.

No health effects result when carboxyhemoglobin levels are under 4% to 5% in healthy adults. Carboxyhemoglobin levels of 2 to 3% may have negative effects on those with cardiovascular disease or other sensitivity. See, Environmental Health Criteria 13, Carbon Monoxide, World Health Organization, Geneva, Switzerland (1979), p. 15.

The US Occupational Safety and Health Administration's air contaminants regulation, 29 CFR Sec. 1910.1000, lists 50 ppm and approximately 55 mg/m^3 of CO as the 8-hour Time Weighted Average of exposure for the substance. 29 CFR Sec. 1910.1000.

By contrast, as explained below, the worst case estimated intake of CO attributable to AT2001 is 1.88 mg CO/meal.

The US FDA has not established an Acceptable Daily Intake for CO. Nevertheless, CO exposure, at levels much higher than those attributable to AT2001, for decades has been permitted within the existing FDA and USDA food additive regulatory provisions:

- Wood smoke ("smoke flavoring"), conventionally including CO as a component, is permitted by regulation as an ingredient in meat and poultry products pursuant to 9 CFR Secs. 318.7(c)(4)[meat], 381.147(c)(4)[poultry], 424.21(c).
- The specifications for Combustion product gas in 21 CFR Sec. 173.350 permit CO up to 4.5 percent by volume in such gases, which may be used in the processing and packaging of beverages and other foods except fresh meats, to remove and displace oxygen. Such gases are commonly used to package fruits and vegetables.

- In 2000, FDA responded favorably to GRAS Notice 000015 from Hawaii International Seafood, Inc. for the use of tasteless smoke, before freezing of tuna, as a preservative, specifically to preserve taste, aroma, texture and color. GRAS Notice (GRN) No. 000015, March 10, 2000. CO is a primary component of conventional smoke, which that Notice asserts is Generally Recognized As Safe based on decades of safe use in a variety of foods, which uses are recognized by FDA and incorporated into numerous food standards that permit smoking of cheeses. CO is also a primary component of tasteless smoke, along with nitrogen, oxygen, carbon dioxide and methane. The tasteless smoke is used to impart a "preservative effect." As noted in FDA's March 10, 2000 letter about the GRAS Notice, "In Hawaii International's view, tuna treated with tasteless smoke and tuna treated with conventional smoke contain comparable levels of carbon monoxide, carbon dioxide, hydrocarbons, and phenols." FDA's letter notes that it "has no questions at this time" regarding Hawaii International's conclusion that the use described is GRAS, though, in keeping with current regulatory practice, it had not made its own determination.

CO is listed as a reproductive toxicant by the State of California pursuant to its Safe Drinking Water and Toxic Enforcement Act of 1986 ("Proposition 65"). California law contemplates that exposures to listed reproductive toxicants will be accompanied by a warning, unless the exposure is less than 1/1000th of an established no observable effect level. Cal. Health and Safety Code, Sec. 25249.6. No such level has been established for CO. Almost without question, though, any such future level (which will have a dubious connection to safety principles in any event, due to the design of Prop. 65), will be more than 1,000 times any possible exposure that could result from AT2001. The worst-case potential exposures from AT2001 are tiny fractions of the established occupational and environmental exposure levels (see below), which themselves are certain to be well below any level at which reproductive toxicity is ultimately is deemed to result.

Effects on Fresh Meat and Consumption

Analysis of the AT2001 system makes plain the lack of any safety issue from consumption of treated meats. Additionally, similar technologies employing CO as part of a modified atmosphere gas mixture analyzed the technologies for effects on meat in terms of microbial load and organoleptic

properties including color, and for the safety of consumption of treated meats, specifically, any tendency of the consumed meat to expose consumers to levels of carboxymyoglobin. Further important evidence is obtained from examination of the actual experience since 1985 in Norway of packaging fresh red meats in 0.3 – 0.5 % CO for retail.

Safety: Effects on carboxymyoglobin levels

Consumption of meat treated with AT2001 is not expected to result in any measurable levels of carboxymyoglobin in the blood of those who consume treated meat.

An Estimated Daily Intake ("EDI") of CO attributable to the AT2001 use can be calculated as follows. First, we assume the following reasonable values for the exposure parameters:

(1) An AT2001 bag contains 1.5 L modified atmosphere with a CO concentration of 0.4%, that is equivalent to approximately 0.006 L of CO in the bag (= 6 mL CO).

(2) At 28 g CO per mole and approximately 22.4 L per mole, the mass of CO per unit volume may be calculated: (28 g/mol)/(22.4 L/mol) = 1.25 g/L = 1.25 mg/mL.

(3) The AT2001 bag contains 2 lbs (approximately 1.0 kg) of ground meat.

(4) Approximately 30% of the total amount of CO is absorbed into the meat (based on Watts, D.A.; Wolfe, S.K.; Brown, W.D., "Fate of [^{14}C] Carbon Monoxide in Cooked or Store Ground Beef Samples", J. Agric. Food Chem., Vol. 26, No. 1 (1978), pp. 210-214). Therefore, the amount of CO taken up by the meat is [(0.3)*(6 mL/bag)*(1.25 mg/mL)] / [1.0 kg meat/bag] = 2.25 mg CO / kg meat.

(5) If we assume that a person consumes an 8.8 oz steak (250 g = 0.25 kg meat), or ground equivalent, at a single meal[2], that 85% reduction in CO content occurs during cooking, and that 100% of the ingested CO is absorbed, then the maximum amount of CO exposure is (0.15)*(2.2.5 mg CO /kg meat)*(0.25 kg meat/meal) = 0.084 mg CO/meal.

Next, comparison may be made of the of the consumer EDI for CO to that amount inhaled during an 8-hour period at the EPA's National Ambient Air Quality Standard ("NAAQS") level. 40 CFR Sec. 50.8, National primary ambient air quality standards for carbon monoxide:

[2] Note that this is a conservative assumption. The EDI of beef for the 90th percentile intake per user is 139.2 g/d based on the most recent USDA national survey of food intake by individuals. Pactiv chose to use a larger value for beef consumption to simulate a typical to above-average consumption incident rather than an average over all meats.

(1) The EPA NAAQS of 9 ppm CO is equivalent to approximately 10.4 mg CO per m^3 air.

(2) The typical person breathes 15 m^3 air per day or approximately 5 m^3 air per 8-hours.

(3) The exposure under these circumstances may be calculated as follows: (10.4 mg/m^3)*(5 m^3/8-hr) = 52 mg CO / 8-hr.

Thus, the ingestion of residual CO from the cooked meat is a small fraction of the exposure level at EPA's NAAQS: (0.084 mg) / (52 mg) = 0.0016 = 0.16%.

Even using worst-case assumptions, the expected exposure is exceedingly low. Assuming again 6 mL/bag; 1.25 mg/mL is the mass of CO per unit volume; and assuming 2 lbs (approximately 1.0 kg) of ground beef per bag; and consumption of an 8.8 ounces of meat; but now assuming no reduction in CO during cooking, and 100% of CO absorbed, the maximum theoretical CO exposure is 1.88 mg CO/meal [(7.5 mg CO/kg meat)x(0.25 kg meat/meal) = 1.88 mg CO/meal.

In turn, using this figure to compare with the EPA's NAAQS, we find that ingestion of residual CO from the cooked meat would be at worst 3.6% of EPA's NAAQS. [(1.88 mg)/(52 mg) = 0.036 = 3.6%)

The calculated, worst case consumer EDI for CO may be compared to that amount inhaled during an 8-hour period at the American Conference of Governmental Industrial Hygienists ("ACGIH") Threshold Limit Value ("TLV"). Documentation of the Threshold Limit Values and Biological Exposure Indices, p. 23, ACGIH, 1330 Kemper Meadow Drive, Cincinnati, Ohio.

(1) The ACGIH TLV is 25 ppm CO is equivalent to approximately 28.9 mg CO per m^3 air.

(2) The typical person breathes 15 m^3 air per day or approximately 5 m^3 air per 8-hours.

(3) The exposure under these circumstances may be calculated as follows:

$$(28.9\ mg/m^3)*(5\ m^3/8\text{-}hr) = 145\ mg\ CO\ /\ 8\text{-}hr.$$

Thus, the ingestion of residual CO from the cooked meat is merely 1.3% of the exposure level at ACGIH TLV ((1.88 mg) / (145 mg) = 0.013 = 1.3%)

Finally, the calculated worst case consumer EDI for CO may be compared to that amount inhaled during an 8-hour period at the OSHA PEL:

(1) The OSHA PEL is 50 ppm CO is equivalent to approximately 58 mg CO per m^3 air.

(2) The typical person breathes 15 m^3 air per day or approximately 5 m^3 air per 8-hours.

(3) The exposure under these circumstances may be calculated as follows:

$$(58\ mg/m^3)*(5\ m^3/8\text{-}hr) = 290\ mg\ CO\ /\ 8\text{-}hr.$$

Thus, the ingestion of residual CO from the cooked meat is 0.65% of the exposure level at OSHA PEL. ((1.88 mg) / (290 mg) = 0.0065 = 0.65%).

Thus, the consumer EDI of CO from a eating meat packaged in the Active Tech 2001 bag is a small fraction of any of the currently allowed exposures by authoritative agencies. As these various limits were established to protect individual safety and health, it is plain that the worst case exposures that may result from AT2001 present no safety concerns whatsoever.

In the 1997 study, "Technological, hygienic and toxicological aspects of carbon monoxide used in modified-atmosphere packaging of meat" Trends in Food Science and Technology, September 1997 [Vol. 8], pp. 307-312, Sørheim, et al. concluded that meat packaged and displayed in an atmosphere combining 60 to 70% carbon dioxide, 30 to 40% nitrogen, and less than 0.5% CO "will result in only negligible levels of carboxyhemoglobin in the blood."

The authors note that there was sparse information in published literature on exposure to CO after consumption of meat treated with CO gas. They note that "the inhalation of air containing CO at a level of 55 mg per m^3 (the acceptable level in working environments in the USA) would provide a COHb level for a prolonged time period (hours) of at least 14 times that of the level reached temporarily on the consumption of 225 g of meat that has been packaged in CO at the saturation level for myoglobin." That estimate assumed saturation of meat myoglobin and hemoglobin was maximal and the transfer of CO from the gastrointestinal tract to the blood was 100%. Sørheim, et al. (1997), p. 310. The authors concluded, "Consequently, even for such a "worst case" scenario, the treatment of meat with CO gas appears to contribute very little to COHb levels, relative to levels that are considered safe in the working environment." Sørheim, et al. (1997), p. 310.

The authors report that "CO is lost from previously CO-treated meat during storage in the absence of CO, with a half life of ~3d." Sørheim, et al. (1997), p. 310. As these fresh meats are to be cooked before consumption, CO lost during cooking is also relevant. The authors report that "When the beef was cooked at 195° C, only 0.1 mg of CO remained

per kg of meat. The loss of CO amounted to ~85%." Sorheim, et al. (1997), p. 310.

The authors also compared CO exposure from the air and estimated exposure from CO-treated meat. Their comparative table is shown below.

Table 5. Theoretical Uptake of Carbon Monoxide (CO) in Blood

Exposure method	**CO intake in 1 h**	**CO intake in 8 h**
Lungs ($15m^3$)	24 mg x 0.625 = 15.1 mg	9.2 mg x 5 = 46.0 mg
Meat (250 g CO treated)	0.025 mg	0.025 mg

Sørheim, et al. (1997), p. 311, Table 5.

Part of the authors' analysis was the premise that absorption of CO from the gastrointestinal tract into blood will in all probability be less effective than absorption from the lungs. The authors summarized the comparison as follows:

> In order to prevent a maximum COHb level in the blood of 1.5% being exceeded, the CO concentration in air for a 1h period of moderate physical activity should not exceed 24 mg/m^3, or 9.2 mg/m^3 in 8h (according to Table 4). In contrast, the consumption of meat that had been treated for 3d in an atmosphere containing 1% CO yielded ~0.1 mg of CO per kg of meat on storage and cooking.

Sørheim, et al. (1997), p. 310, citing Watts, D.A.; Wolfe, S.K.; Brown, W.D., "Fate of [^{14}C]Carbon Monoxide in Cooked or Stored Ground Beef Samples", *J. Agric. Food Chem.*, Vol 26, No. 1 (1978), pp. 210-214.

The authors calculate that CO intake in 1h through the lungs taking in 15m^3 per day would result in 15.1 mg of CO, as compared with 0.025 mg of CO from intake of 250 g of CO treated meat. In 8 hours, the authors say the lungs will take in 46.0 mg, and the figure for meat would still be 0.025 mg. As the authors conclude,

> Estimates detailed above indicate that, even assuming an improbable 100% absorption of CO from the gastrointestinal tract into the blood, the consumption of meat that has been treated with 1% CO will result in COHb levels that are negligible (approximately 3 orders of magnitude lower) compared with those resulting from exposure in the working environment to CO at an acceptable level. Consequently, it is highly improbable that CO exposure from meat packaged in an atmosphere containing up to 0.5% will represent a toxic threat to consumers through the formation of COHb.

Sørheim, et al. (1997), p. 310.

In another published report, the storage life and characteristics of meats packaged in a modified atmosphere including 0.4% CO were studied, but under circumstances distinguishable from AT2001. Sørheim O; Nissen, H; Nesbakken, T, "The Storage Life of Beef and Pork Packaged in an Atmosphere With Low Carbon Monoxide and High Carbon Dioxide", 52 Meat Science 157-164 (1999). In the study, the meats were packed in

modified atmosphere into retail-ready packages. This study examined off odor and microflora, as well as color, comparing the 0.4% CO/ 60% CO_2 /40% N2 gas mixture with a gas mixture of 70% oxygen and 30% CO_2.

Among the points made by these authors was that there is sometimes an objection raised against using CO in retail ready meats because "the colour stability can exceed the microbiological shelf life, with the risk of masking spoilage of the meat." Sørheim, et al. (1999), p. 163. (Citing Kropf, D.H. (1980), "Effects of retail display conditions on meat colour", *Proceedings of the Reciprocal Meat Conference, 33, pp. 15-32.*) The authors assert that in those circumstances, consumers would need to rely on off odors to evaluate microbiological conditions of meat. In addition, they caution, "When a MA with CO is applied commercially, it is important to have a proper control of hygienic condition of the meat raw materials and the chill chain temperatures." See Sørheim, et al. (1999), p. 163.

AT2001, by contrast, presents no such similar problems or needs for caution. AT2001 does not mask spoilage of the meat. AT2001 does not involve use of a modified atmosphere including CO in the retail package. Moreover, as noted below, Pactiv's own commissioned experimentation with AT2001 demonstrates that AT2001 retail packages will deteriorate in color beginning almost immediately after removal of the modified

atmosphere, and that microbial load will not reach unsafe levels while the color of AT2001 meat is still acceptable to the consumer.

Safety: The Norwegian experience

In Norway, CO has been used to package fresh meats, even at retail, since 1985, with commercially satisfactory and safe results.

The 2000 submission by the Norwegian Meat Cooperative and Norwegian Independent Meat Association to the EU Commission seeking Europe-wide approval of the use of CO, "Application For Assessment Of The Food Additive Carbon Monoxide (CO) Prior To Its Authorization", is Attachment 3. The evaluation undertakes a detailed analysis of the CO exposure expected through the described packaging use. See section entitled "IV. Report by Tore Aune: "Fresh Meat in Consumer Packaging-A Toxicological Evaluation of the Use of Up to 0.5% CO in a Gas Mixture".

As the Norwegian risk assessment analysis concludes, assuming a worst-case exposure of 0.1 mg/kg from consumption of 250 grams of heated CO-treated meat, CO intake can be expected to be 0.025 mg in 1 hour or even after 8 hours. Attachment 3, p. 000154. The cited study, Sørheim, et al. (1997), utilized meat that had been treated with 1% CO. According to the authors, to stay under maximum blood levels of carboxyhemoglobin of 1.5%, "the CO concentration in the air must be 24

milligrams per mg/m^3 for 1 hour at moderate physical activity at 9.2 mg/m^3 for 8 hours...." Attachment 3, p. 000154. Assuming an adult inhales 15 m^3 per 24 hours, this translates to 15.1 mg of CO taken in 1 hour, or 46.0 mg of CO taken in 8 hours. This is in dramatic contrast to the miniscule amount expected to be ingested through meat. The Norwegian authors conclude, "From a health perspective, the use of CO in concentrations below 0.5-1% for fresh meat thus represents no toxicological risk." Attachment 3, p. 000155.

Safety: Exposure in environment

As a basis for comparison, the possible effect on ambient CO concentration associated with the release from a typical AT2001 barrier bag was estimated. A typical AT2001 barrier bag contains approximately 1.5 liters of modified atmosphere with a CO concentration of 0.4 percent, which is equivalent to approximately 0.006 liters of CO within the bag. On a mass basis, this volume of CO is equivalent to approximately 0.0075 g (7.5 mg) CO per bag.

Consider the possible use of the bag for storage of meat prior to retail display (e.g., at a supermarket). Any unassociated CO within the bag, it can be assumed, would be released to the meat processing area when the bag is opened, resulting in possible exposure by the employee(s) to the

released CO. The extent of such exposure is dependent on several factors, including the size of the meat processing area, air-mixing within the area and between adjacent areas, the number of bags opened, and the amount of free CO unassociated with the meat in the package. For these calculations, it has been conservatively assumed that none of the CO has become associated with the meat and is therefore all free to the ambient atmosphere upon opening of the package.

Assume, however, that the air volume within a meat processing area may reasonably range from 150 m^3 to 1,500 m^3, which would represent several hundred to several thousand square feet of processing area. If each bag introduces 7.5 mg CO to the air within the processing area, the corresponding concentration of CO in air would be in the range of 0.005 mg/m^3 to 0.05 mg/m^3, assuming conservatively that there is no air exchange between the processing area and other rooms or the outdoors. Thus, to exceed the occupational safety standard (i.e., threshold limit value, or TLV) of 25 ppm (29 mg/m^3), 580 to 5,800 1.5 liter bags would need to be opened within an 8-hour period. As noted above, this assumes no mixing with other areas of the building or with outdoor air.

Thus, applying the reasonable assumption that the air volume within the processing area will be exchanged with external air once per hour,

opening of 580 to 5,800 bags per hour would be required to exceed the TLV, or 4,600 to 46,000 bags per work day. The number of bags opened within a given processing area will be a function of the size of the processing area, to a given extent, but is unlikely to even approach the number of bags required to result in air concentrations at the TLV. Actual concentrations in the work area of a secondary processing facility would likely be one to two orders of magnitude below the standard. Thus, opening of bags within a work area will not alter significantly the environmental exposure to CO.[3]

Regardless, the opening of the bags does not alter significantly the environmental exposure to CO. This action qualifies for a categorical exclusion from preparation of an environmental assessment pursuant to 21 CFR Sec. 25.32 (i), which provides an exemption for, in pertinent part, "Approval of a ...GRAS affirmation petition...." 21 CFR Sec. 25.32(i). The regulation makes no specific mention of the GRAS Notice procedure, but similar treatment is warranted for a GRAS Notice. (We also note that CO as used here also qualifies for exclusion under 21 CFR Sec. 25.32(r), as CO "occurs naturally in the environment" and the noticed use "does not alter significantly the concentration or distribution of the substance, its

[3] As an aside, there is no reason to expect any difficulty achieving compliance with the OSHA Threshold

metabolites, or degradation products in the environment." 21 CFR Sec. 25.32(r).)

Corroborative information about AT2001

The specific AT2001 system has been thoroughly tested to confirm that it results in the expected limited exposures to CO, and has no adverse effects on the treated meats. A study of meats treated with AT2001 commissioned by Pactiv examined its effects on initial product color, stability of color during display, and the central safety consideration of the relationships between color deterioration and microbial populations.

The study, conducted by faculty of the Department of Animal Sciences & Industry of Kansas State University, Manhattan, Kansas, examined steaks from three cuts of beef (strip loin, tenderloin, and inside round steaks), as well as ground beef. The study report is Attachment 4. The meats were packaged in AT2001 atmosphere, then stored at 35° F or 43° F for up to 35 days. Cuts were then placed under simulated retail conditions by being removed from the AT outer package and displayed at 34° F until their color approached consumer unacceptability. Comparisons

Limit Value at plants using the AT2000 technology to fill bags. Experimental use of an exhaust hood over the machinery has resulted in no measurable increase in CO ppm levels near the line.

were made to similar products that had been exposed to oxygen but not CO.

Among the study's conclusions were:

(1) Color: AT2001 *system resulted in products that were equally red to products packaged with traditional oxygen permeable overwrap.* When the AT2001 outer bag was removed, the product's conversion to oxymyoglobin occur red in 60-90 minutes and then had a typical bright red color. Visual appearance was improved, especially in the tenderloin and inner part of the inside round steaks, throughout display. Color deterioration compared well to baseline products exposed to oxygen. For tenderloin and inside round steaks, and to a lesser degree for ground beef, display time was increased only slightly in the AT2001 samples.

(2) Bacterial growth: Bacterial growth was neither encouraged nor suppressed by the addition of CO to the ActiveTech™ *gas blend (nitrogen and carbon dioxide)*, although microbial growth curves changed in slope and exponential growth according to the environment in the packages. Aerobic bacteria and facultative anaerobes followed typical patterns of growth according to environmental conditions.

(3) Spoilage indicators: CO neither masked spoilage, nor extended color life beyond the point of wholesomeness (i.e., the point of microbial soundness).

A summary of the study follows.

A random selection of all steaks and ground beef packaged using oxygen-permeable polyvinyl chloride ("PVC") film were placed in display to serve as a baseline for color and microbiological comparisons. Products were expected to have the lowest microbiological load and ideal color stability using traditional packaging and display conditions for products exposed only to atmospheric oxygen. The inherent muscle chemistry responsible for good color life also was optimal. If the product exposed to CO were to have extended meat color life, then it will be compared to the baseline "control" with the "best" possible color.

To measure color changes, visual scores were considered the "standard" with instrumental color being discussed relative to its agreement or disagreement with the visual panel, ie, did the objective measurements confirm what the color panel saw? Visual scores of ≥ 3.5 were considered borderline acceptable. When samples reached this discoloration, they were removed from display. Normally, a* values (higher values indicate more redness) are highly correlated to visual appraisal.

Inside round steaks typically are two-toned in color. The inner portion (ISM) is much less color stable than the outer portion (OSM). These portions were scored separately since one portion may have acceptable color while the other has unacceptable color that would be discriminated against by consumers resulting in the whole cut being judged unacceptable in color. The effects of CO on this bi-colored muscle were needed to confirm that color was not excessively extended in either portion.

Average fat and moisture contents of the ground beef were 19.5 and 61.6%, respectively. The pH of both intact muscles and the ground beef ranged from 5.3 to 5.7. The initial aerobic plate counts and lactic bacteria counts for all products were relatively low and indicative of good microbial quality of the raw materials and good sanitation. Furthermore, coliforms and *E. coli* were below the detection limit throughout the study.

The color of ground beef and steaks entering display (after MAP storage at 2 temperatures) was an attractive, typical red color. Although there were several significant differences in visual scores and a* values (Attachment 9, Table 2 and Figures 1-10 at day 0) for product CO vs. baseline cuts, the variation in color was usually within $\pm$ 0.5 of a color score.

Color results: In general, the initial color of product exposed to CO was very similar to the color of steaks from the baseline display (never exposed to CO). When differences occurred, they were more related to either storage temperature or postmortem age of the product.

Panelists did not consider the color of product exposed to CO atypical. Cuts exposed to CO generally appeared more uniformly bright-red and would be expected to have high consumer appeal. These results were expected, as CO is known to preferentially form a ligand with the colored pigment (myoglobin) in meat resulting in a more intense red pigment known as carboxymyoglobin.

In the AT2001 system, Pactiv uses a low level of 0.4% CO, and obtains a red color very similar to the normal red oxymyoglobin pigment of fresh meats exposed to oxygen.

Color stability results: A critical next question was whether the carboxymyoglobin formed on the surface was more stable than the oxymyoglobin formed in baseline product. Further, did the carboxymyoglobin deteriorate in a predictable way that consumers could continue to use visual color to judge freshness or potential spoilage?

Product exposed to CO during MAP storage had color deterioration during display. (See visual panel scores (Attachment 4, Figures 1-5) and

instrumental color (a* values, Attachment 4, Figures 6-10).) As expected, visual scores increased (color deteriorated) and a* values decreased (loss of redness) as days in display increased. In several instances, color appeared to improve late in display – as indicated by a decrease in visual scores (see ground beef, strips loins and tenderloins at 43°F). These decreases were not a return of redness, but resulted from removal of discolored packages the preceding period, leaving product with less overall discoloration remaining in the case.

In general, the color deterioration profiles followed an expected pattern. Namely, the freshest product (baseline packages) had the most stable, red color and the most days in display needed to reach borderline discoloration of all treatments. (Attachment 4, Table 3 scores to 3.5) Exceptions occurred for the inside portion of the inside round and tenderloin products, where the product exposed to CO had slightly more stable color than the baseline product (Attachment 4, Table 3). These two muscle areas are well known by retailers as having short color life. Thus, CO appeared to improve color life when the inherent muscle chemistry desired for color was limited.

For product from MAP, the longer the storage time, the faster the deterioration, especially at the higher storage temperature (Attachment 4,

Tables 2 and 3). For packages stored at 43°F, which was a mildly abusive temperature, color deterioration would be expected to accelerate. This phenomenon also is illustrated in Attachment 4, Figures 1-10.

There was no evidence the color shelf life was unexpectedly lengthened by exposure of meat to CO in MAP. Changes in a* values (and other instrumental measures of color not shown) followed the same pattern of color deterioration observed by the visual panelists.

Color and microbial data: Initial, pre-display microbiological data suggested that the raw materials were fresh and processed using good hygienic practices. For intact cuts, lactic acid bacteria, generic *E. coli*, and total coliform counts were below the detection limit of 1.76 colony forming units (CFU)/in^2. Initial, pre-display aerobic plate counts ("APC") for intact muscles ranged from 1 to 1.63 $\log_{10}$ CFU/in^2 . Post-display counts were higher ($P<0.05$) than pre-display APC which was an increase in bacterial proliferation and typical deterioration. However, all product had sufficient microbes to be susceptible to spoilage.

Baseline products were pulled from display when the visual panel scores reached ≥ 3.5. However, the APC did not exceed 5 $\log_{10}$ CFU/unit as shown in Attachment 4, Figures 15-18. Furthermore, off-odor scores for

product at end of display (Attachment 4, Table 3) ranged from no to slight off odor.

Thus, color life in this base population did not exceed microbial soundness, which is generally accepted as < 100 million CFU/g hamburger (<1 x 10^8). (Principles of Meat Science, 3d Ed., Hedrick, H.B.; Aberle, ED, Forrest, JD; Judge, MD; Merkel, RA, Eds, Kendall/Hunt Publishing Co., Dubuque, Iowa.

Similar trends in microbial growth occurred in post-displayed samples stored in MAP compared to baseline products. Microbial patterns for product deterioration are shown in Attachment 4, Table 4 and Figures 11-18. Products stored under MAP at a slightly abusive temperature showed, as expected, a more rapid increase ($P<0.05$) in microbial counts compared to samples stored at 35°F. For post-MAP (pre-display) and post-display samples, APC were higher at 45°F than 35°F (Table 4), and during the later days of storage at the higher temperature, differences were more obvious. Significant changes ($P<0.05$) occurred in all cuts and ground beef with the exception of semimembranosus muscle. Counts for the SM muscle were lower than expected and no significant changes occurring until day 35 of MAP storage. This suggests that quality products that have been handled

in a sanitary fashion can be stored in the AT2001 system up to 35 days without comprising microbial quality.

The APCs for intact strip loin and tenderloin steaks stored at 35°F were lower ($P<0.05$) on all days of display on days 21 and 35 post-MAP than steaks stored at 43°F (Attachment 4, Figures 12 and 14). Although products did not show a difference in APCs 7 days post-MAP, those products stored at the higher temperature (43°F) were more inferior 21 and 35 days post-MAP.

One goal of this research was to see if the color of CO-treated meat might mask spoilage. Visual color scoring was considered as the "standard" for determining the time to remove products from display. Because the visual panel scores were the deciding factor for length of shelf life, the interdependence between visual color and APC, LAB, and odor were considered quite important.

Attachment 4, Figures 19-21 show aerobic and lactic bacterial growth and odor scores at the end of display plotted against their corresponding visual color scores. All data observations were summed over storage temperature, storage time, and product type and plotted in one graph. If color masked spoilage, then there would be multiple points in the upper left

quadrant of the plot, the area represented by unacceptable microbial counts and off odors but with acceptable color (i.e., scores <3.5).

This did not occur with any frequency in any of the three plots. Thus, it does not appear that exposure of meat to CO during extended (up to 35 days at either 35° or 43°F) caused meat color to hide spoilage.

e. Statement of availability of information

Notifier has relied on published studies and generally accepted scientific data and information as the basis of its conclusions, and those of its panel of experts, about the safety and the general recognition of a modified atmosphere system for meat incorporating 0.4% CO in the gas mixture.

II. Identity of notified substance

1. Chemical name: Carbon monoxide

2. Chemical Abstracts Service: **630-08-0**

3. Composition Specifications for food-grade material: The CO employed in this system is to be of suitable purity for food contact. Specifically, this will mean a 99.99% minimum purity, as supplied by Pactiv's commercial gas supplier, Haun Welding Supply, Inc., 6481 Ridings

Road, Syracuse, NY 13206. Attachment 5. The supplier's CO meets the following specifications, and will be referred to as "commercial grade":

Component	Specification
Carbon Monoxide	99.99% min.
Oxygen	≤ 0.5 PPM
Nitrogen	≤ 10 PPM
Carbon Dioxide	≤ 20 PPM
Methane	≤ 5 PPM
Ethane	≤ 1 PPM
Propane	≤ 1 PPM
Dimethyl Ether	≤ 1 PPM
Hydrogen	≤ 1 PPM
Moisture	≤ 1 PPM

4. Properties:

Relative molecule mass	28.01
Critical point	–140.2 °C at 34.5 atm (3.5 MPa)
Melting point	–205.1 °C
Boiling point	–191.5 °C
Density, at 0 °C, 1 atm	1.250 g/litre
at 25 °C, 1 atm	1.145 g/litre
Specific gravity relative to air	0.967
Solubility in water at 0 °C, 1atm	3.54 ml/100 ml
at 25 °C, 1 atm	2.14 ml/100 ml
at 37 °C, 1 atm	1.83 ml/100 ml[a]
Conversion factors:	
at 0 °C, 1 atm	1 mg/m^3 = 0.800 ppm[b]
	1 ppm = 1.250 mg/m^3
at 25 °C, 1 atm	1 mg/m^3 = 0.873 ppm
	1 ppm = 1.145 mg/m^3

[a] Value obtained by graphic or calculated interpolation (Altman et al., 1971).
[b] Parts per million by volume

5. Analyses: ASTM D1946, "Analysis of Reformed Gas by Gas Chromatography (GC) with Thermal Conductivity Detection (TCD)", may be utilized to measure the quantity of CO present in gas mixtures. A copy of the method is Attachment 6.

III. Self-limiting levels of use

Studies of modified atmospheres for packaging meat that contained both higher and lower levels of CO have established that the 0.4% used in the AT2001 system both has advantageous characteristics and avoids disadvantages seen with lower or higher levels. A CO level of 0.4% is sufficient to produce stable, cherry red color, (Sørheim, et al. (1997), and use of CO through retail display time may result in masked spoilage.

IV. Basis of GRAS determination.

Pactiv believes its use of CO is GRAS based on scientific procedures, 21 CFR Sec. 170.30(b), and convened a panel of experts qualified by scientific training and experience to evaluate the safety of food, food additives and food ingredients. The experts have reviewed and evaluated the publicly available information summarized in this GRAS Notice. Their testimonial

letters are attached as Attachments 7 through 10. The above discussion and citations to generally available accepted scientific data, information, methods and principles relied upon, together with the anticipated consumption levels for both CO and meat treated with CO, provide ample basis to conclude that the use of CO at 0.4% in a modified atmosphere for packaging fresh meats is both safe and generally recognized as such by qualified experts.

The panel consisted of the following experts, whose GRAS opinions and curricula vitae are attached as attachments 7 through 10.

1. Daren Cornforth, Ph.D.
   Professor
   Department of Nutrition and Food Sciences
   Utah State University
   750 N. 1200 E.
   Logan, Utah 84322-8700

Dr. Cornforth is a professor in Nutrition and Food Sciences at Utah State University, Logan, Utah, and received his Ph.D. in food science and human nutrition from Michigan State University. He has performed extensive research and published multiple articles on the subject of meat color.

2. Vasilios Frankos, Ph.D.
   Principal
   Environ Corp.
   4350 N. Fairfax Dr.

Suite 300
Arlington VA 22203

Dr. Frankos is a Principal at ENVIRON corporation, Arlington, Virginia, a scientific consulting firm, and has over 20 years of experience in the toxicological and pharmacological evaluation of data used to assess the risks posed by foods, food additives, and other substances. He holds a Ph. D. from the University of Maryland Pharmacy School in Pharmacology and Toxicology.

3. Melvin C. Hunt, Ph.D
Professor
Weber Hall
Dept. of Animal Sciences and Industry
Kansas State University
Manhattan, KS 65506

Dr. Hunt is a professor of food science at the Department of Animal Sciences and Industry at Kansas State University, Manhattan, Kansas. He received his Ph.D. in food science at the University of Missouri. Among his many research projects and publications are multiple studies relating to meat color and the effects of various environments on meat color.

4. Oddvin Sørheim, Ph.D.
Senior Research Technologist
MATFORSK – Norwegian Food
Research Institute
Osloveien 1

N-1430 Ås
Norway

Dr. Sørheim is a Senior Research Technologist at the Norwegian Food Research Institute, Osloveien, Norway. He received his Ph.D. in food science from the Agricultural University of Norway, and has performed extensive research and industry consultation, and published numerous articles on meat, including extensive experience with the use of CO in modified atmosphere packaging of meat.

Pactiv is not aware of any reports of investigations that are inconsistent with the GRAS determination relating to the use described.

Conclusion

Based on all the above information, Pactiv Corporation has concluded that its use of 0.4% CO within the AT2001 modified atmosphere system for packaging fresh meat is Generally Recognized as Safe within the meaning of 21 U.S.C. Sec. 321(s).

Sincerely,

Eric F. Greenberg

Attachments:

Attachment 1	Authorization letter from Pactiv Corporation for representation by Eric F. Greenberg
Attachment 2	Photographs of meats treated with ActiveTech™ and ActiveTech™ 2001
Attachment 3	"Application for Assessment of the Food Additive Carbon Monoxide (CO) Prior to its Authorization", Norwegian Meat Cooperative; Norwegian Independent Meat Association (1999)
Attachment 4	"Evaluation of Beef Steaks and Ground Beef in the Pactiv ActiveTech™ Packaging System: Effects of Carbon Monoxide in the Package Atmosphere", Hachmeister, K; Hunt, M.; Milliken, G; May 2001.
Attachment 5	Certificate of Conformance, Carbon monoxide, Haun Welding Supply, Inc., Syracuse, New York, May 8, 2001.
.....	
Attachment 6	"Standard Practice for Analysis of Reformed Gas by Gas Chromatography", ASTM D 1946-90 (Reapproved 2000).
Attachment 7	Daren Cornforth, Ph.D., letter and curriculum vitae
Attachment 8	Vasilios H. Frankos, Ph.D., letter and curriculum vitae
Attachment 9	Melvin C. Hunt, Ph.D., letter and curriculum vitae
Attachment 10	Oddvin Sørheim, Ph.D., letter and curriculum vitae

THIS PAGE BLANK

List of Attachments

Attachments:

Attachment 1 — Authorization letter from Pactiv Corporation for representation by Eric F. Greenberg **000050**

Attachment 2 — Photographs of meats treated with ActiveTech and ActiveTech 2001 ..... **000053**

Attachment 3 — "Application for Assessment of the Food Additive Carbon Monoxide (CO) Prior to its Authorization", Norwegian Meat Cooperative; Norwegian Independent Meat Association (1999) **000058**

Attachment 4 — "Evaluation of Beef Steaks and Ground Beef in the Pactiv ActiveTech Packaging System: Effects of Carbon Monoxide in the Package Atmosphere", Hachmeister, K; Hunt, M.; Milliken, G; May 2001 ..... **000157**

Attachment 5 — Certificate of Conformance, Carbon monoxide, Haun Welding Supply, Inc., Syracuse, New York, May 8, 2001 ..... **000190**

Attachment 6 — "Standard Practice for Analysis of Reformed Gas by Gas Chromatography", ASTM D 1946-90 (Reapproved 2000) **000192**

Attachment 7 — Daren Cornforth, Ph.D., letter and curriculum vitae **000198**

Attachment 8 — Vasilios H. Frankos, Ph.D., letter and curriculum vitae **000205**

Attachment 9 — Melvin C. Hunt, Ph.D., letter and curriculum vitae **000221**

Attachment 10 — Oddvin Sorheim, Ph.D., letter and curriculum vitae **000247**

ATTACHMENT 1

[redacted]

PACTIV
Advanced Packaging Solutions

August 9, 2001

Pactiv Corporation
Technology Center
2651 Brickyard Road
Canandaigua, New York 14424-1026

Division of GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C St, SW
Washington, DC 20204

Re: Authorization of counsel regarding
NOTIFICATION OF CLAIM FOR GENERAL RECOGNITION OF SAFETY OF CARBON MONOXIDE IN A MODIFIED ATMOSPHERE SYSTEM FOR PACKAGING FRESH MEAT

To the FDA:

Please take note that Pactiv Corporation, with headquarters at 1900 West Field Court, Lake Forest, Illinois, 60045, authorizes its attorney, Eric F. Greenberg, 3500 Three First National Plaza, Chicago, Illinois 60602, to represent it and communicate on its behalf in all matters regarding Pactiv's NOTIFICATION OF CLAIM FOR GENERAL RECOGNITION OF SAFETY OF CARBON MONOXIDE IN A MODIFIED ATMOSPHERE SYSTEM FOR PACKAGING FRESH MEAT.

Sincerely,

_____

For PACTIV CORPORATION

By: Vinod K. Luthra
General Manager
New Business Development
2651 Brickyard Road
Canandaigua, New York 14424

[redacted]

Summary regarding Pactiv Corporation

Pactiv Corporation, 1900 West Field Court, Lake Forest, Illinois, is a leading provider of advanced packaging solutions to customers around the world. The company employs 17,000 people in 87 facilities worldwide. Annual revenues exceed $3 billion.

Pactiv manufactures, markets and sells plastic and paper-based consumer products and food/foodservice packaging as well as protective and flexible packaging. Approximately 80% of its revenue comes from products made from different types of plastics, with the balance from paper and aluminum products.

The company's products include a wide range of items for consumers, food processors, supermarkets, foodservice entities, and the construction, automotive, computer, electronic, furniture and durable goods industries. The consumer products are sold under such recognized brand names as Hefty® , Baggies® , Hefty One-Zip® , Kordite™ and E-Z Foil®.

Pactiv further fuels internal growth by developing and commercializing proprietary new products and by designing value-added product-line extensions. In 1998, the consumer products and food/foodservice packaging business introduced over 80 new products and product-line extensions. In the protective and flexible packaging business, where custom design services drive revenues, Pactiv developed over 500 custom product applications in 1998. New product innovations include ActiveTech™ packaging, a proprietary modified atmospheric package used by food processors for case-ready meat.

ATTACHMENT 2

[redacted]

The pages immediately following illustrate:

1. On the top of the photograph on the first page, an example is shown of the structure utilized for both ActiveTech™ and AT2001 incorporating tray, flexible overwrap, outer bag and activated oxygen scavenging sachet.

2. The second page of photographs show examples of ground meat color during the first, second, third and fourth days of display after packaging in (1) Hi-oxygen; (2) AT2001 atmosphere (referred to in captions as "Short Gas", 0.4% CO/35% CO2/64.6 N2), and after being held in that atmosphere for 20 days; and (3) Fresh.

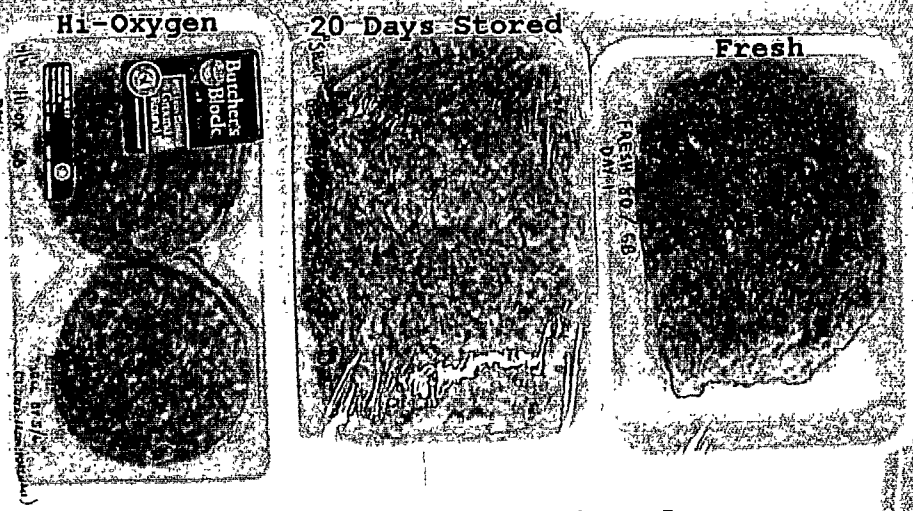
3. The third page of photographs show examples of whole muscle meat (top round steak) color during the first, second, third and fourth days of display after packaging in (1) AT2001 atmosphere (referred to in captions as "Short Gas", 0.4% CO/35% CO2/64.6 N2), and after being held in that atmosphere for 20 days; and (2) Fresh cut.

ActiveTech™
Case Ready Packaging System

For Case Ready Applications

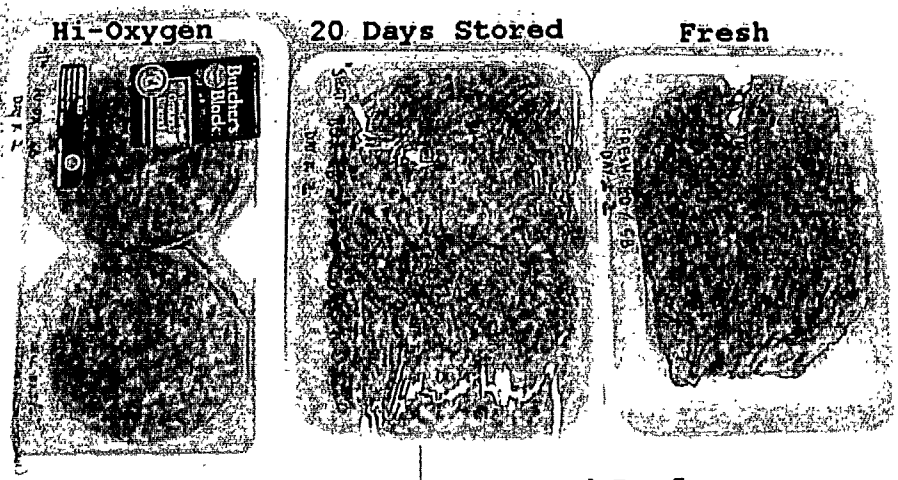
000055

First Display Day



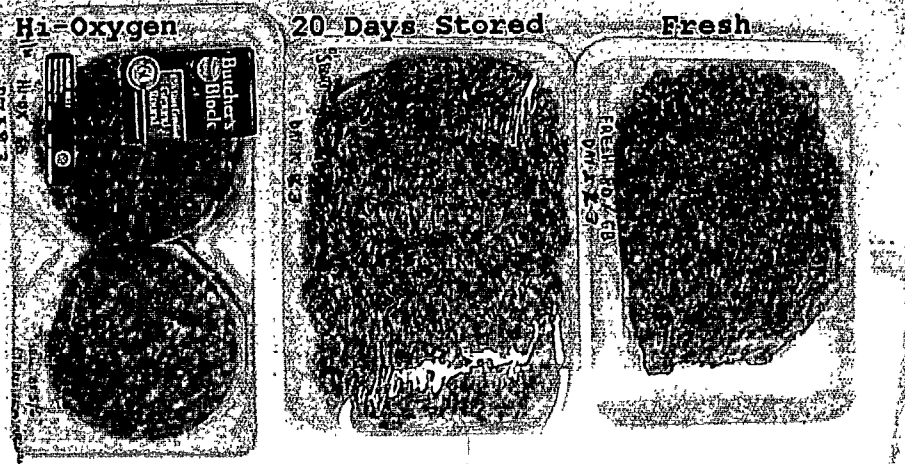
Beef: 80% Lean Ground Beef
Hi-OX, 20 Days Stored in "Short Gas", and Fresh
(0.4% CO/ 35% CO2/ 64.6% N2)

Second Display Day



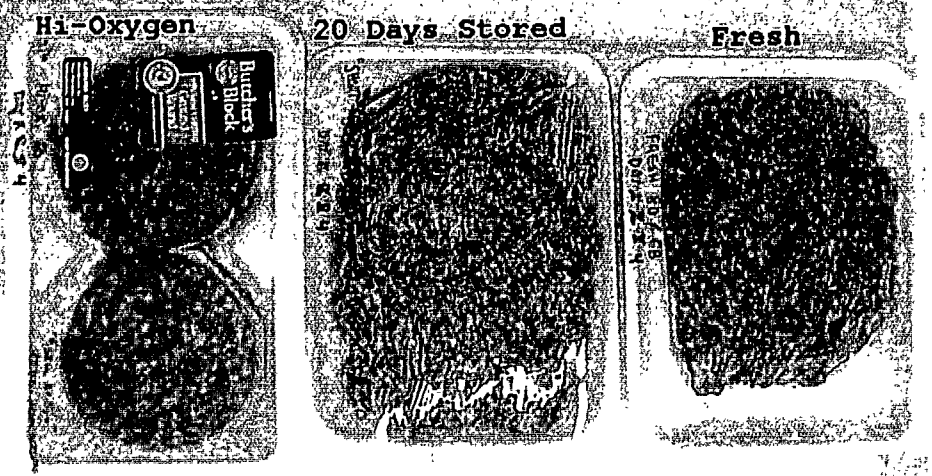
Beef:80% Lean Ground Beef
Hi-Ox, 20 Days Stored in "Short Gas", and Fresh
(0.4% CO/ 35% CO2/ 64.6% N2)

Third Display Day



Beef: 80% Lean Ground Beef
Hi-Ox, 20 Days Stored in "Short Gas", and Freah
(0.4% CO/ 35% CO2/ 64.6% N2)

Fourth Display Day



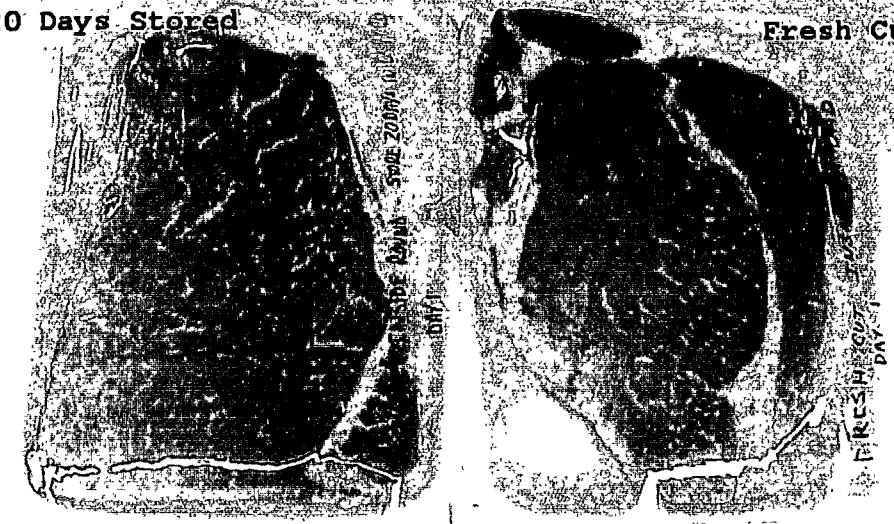
Beef: 80% Lean Ground Beef
Hi-Ox, 20 Days Stored in "Short Gas", and Fresh
(0.4% CO/ 35% CO2/ 64.6% N2)

000056

First Display Day

20 Days Stored

Fresh Cut

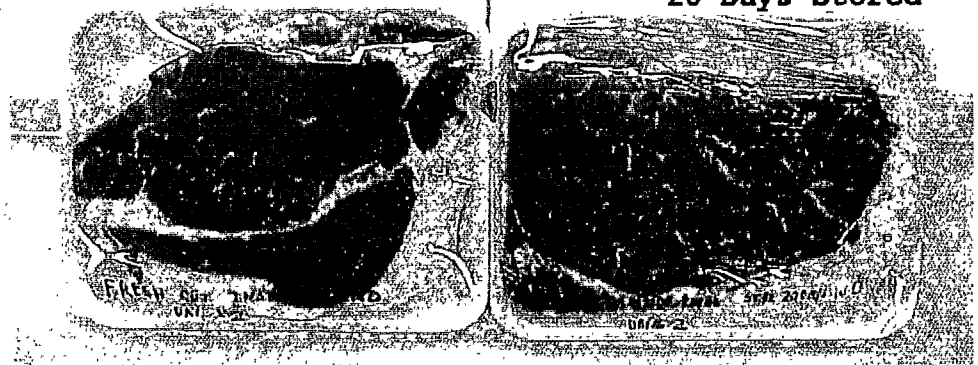


Beef: Top Round Steak
Fresh Cut and 20 Days Stored in "Short Gas"
(0.4% CO/ 35% CO2/ 64.6% N2)

Second Display Day

Fresh Cut

20 Days Stored



Beef: Top Round Steak
Fresh Cut and 20 Days Stored in "Short Gas"
(0.4% CO/ 35% CO2/ 64.6% N2)

Third Display Day

20 Days Stored

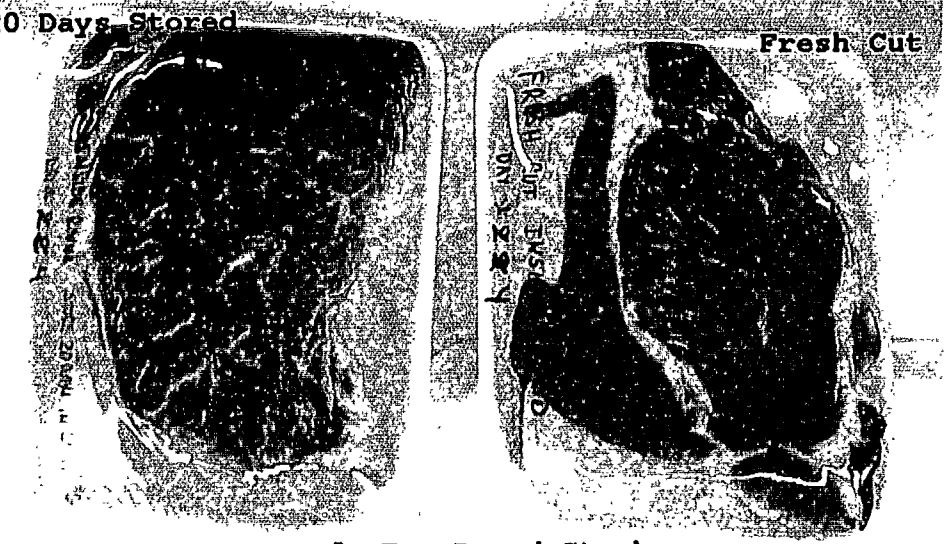
Fresh Cut

Beef: Top Round Steak
Fresh Cut and 20 Days Stored in "Short Gas"
(0.4% CO/ 35% CO2/ 64.6% N2)

Fourth Display Day

20 Days Stored

Fresh Cut



Beef: Top Round Steak
Fresh Cut and 20 Days Stored in "Short Gas"
(0.4% CO/ 35% CO2/ 64.6% N2)

000057

ATTACHMENT 3

[redacted]

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:

Lørenveien 37
P.O.Box 360 Økern
0513 Oslo, Norway

Other means of communication:

Telephone: +47 22 09 21 00
Fax: +47 22 15 59 08

Applicant no. 2:

The name of the applicant:

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

Address:

Karoline Kristiansensvei 2, Fyrstikktorget
P.O.Box 6279 Etterstad
0603 Oslo, Norway

Other means of communication:

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

000059.001

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.

REFERENCES

Bruce, J. (1990) A comparison of American and European markets for the MAP of fresh red meats, in Proc. Int. Conf. Modified Atmosphere Packaging (Day, B.P.F., ed.), Stratford-upon-Avon, Part 2, pp. 1 - 14, Campden Food & Drink Res. Assoc., Chipping Campden, UK

Commission of the European Union (CEU) (1981) Food - Science and Technique. Report from the Scientific Committee for Food, 11th Series, p. 6, Brussels, Belgium

Cornforth, D. (1994) Color - its basis and importance, in Quality attributes and their measurement in meat, poultry and fish products. Adv. Meat Res. - Vol. 9 (Pearson, A.M. & Dutson, T.R., eds.), pp. 34 - 78, Blackie

El-Badawi, A.A, Cain, R.F., Samuels, C.E. and Angelmeier, A.F. (1964) Color and pigment stability of packaged refrigerated beef. *Food Technol.* 18, 159-163

European Commission. Reports of the Scientific Committee for Food. 25th series, Annex II, pp. 17 - 18, 1991

European Commission, Scientific Committee for Food, Minutes of the 103rd meeting, 19 - 20 September 1996, p. 8, 1996

European Parliament and Council Directive (1995) of 20 February 1995 on Food Additives Other than Colours and Sweeteners. Off. J. Eur. Commun. No. L61, 1 - 38, 18.3.95

Gill, C.O. (1988) The solubility of carbon dioxide in meat. Meat Sci. 22, 65 - 71

Gill, C.O. (1996) Extending the storage life of raw chilled meats. Meat Sci. 43 (suppl. issue), S99 - S110

Gill, C.O. and McGinnis, J.C. (1995) The effects of residual oxygen concentration and temperature on the degradation of the colour of beef packaged under oxygen-depleted atmospheres. Meat Sci. 39, 387 - 394

Kropf, D.H. (1980) Effects of retail display conditions on meat color. Proc. Reciprocal Meat Conference 33, 15 - 32

Lanier, T.C., Carpenter, J.A., Toledo, R.T. and Reagan, J.O. (1978) Metmyoglobin reduction in beef as affected by aerobic, anaerobic and carbon monoxide containing environments. J. Food Sci. 43, 1788 - 1792, 1796

Nesbakken, T. *Yersinia species* (2000) In: The microbiological safety and quality of food. Lund, B. M., Baird-Parker, T. C., Gould, G. W. (eds.), Aspen Publishers, Inc., Gaithersberg, MD, USA, pp. 1363-1393.

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.

Nissen, H., Alvseike, O., Bredholt, S., Holck, A. and Nesbakken, T. (submitted) Packaging of grround 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.

Renerre, M. and Labadie, J. (1993) Fresh red meat packaging and meat quality. Proc. Int. Congr. Meat Sci. Technol., 39, 361-387

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

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

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

Sørheim, O., Erlandsen, T., Nissen, H., Lea, P. and Høyem, T. (1997b) Effects of modified atmosphere storage on colour and microbiological shelf life of normal and pale, soft and exudative pork. Meat Sci. 47, 147 - 155

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

Taylor, A.A., Down, N.F. and Shaw, B.G. (1990) A comparison of modified atmosphere and vacuum skin packing for the storage of red meats. Int. J. Food Sci. Technol. 25, 98 - 109

Watts, D.A., Wolfe, S. K., and Brown, W. D. (1978). Fate of [^{14}C] carbon monoxide in cooked or stored ground beef samples. J. Agric. Food Chem., 26, 210 - 214

Wolfe, S.K. (1980) Use of CO- and CO_2-enriched atmospheres for meats, fish, and produce. Food Technol. 34 (3), 55 - 63

WHO (1979) Environmental health criteria. 13. Carbon monoxide. World Health Organization, Geneva, Switzerland

WHO (1987) Air quality guidelines for Europe. World Health Organization Regional Publ., European Series, no. 23

1

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

H. Nissen*, O. Alvseike[1,2], S. Bredholt, A. Holck and T. Nesbakken[2]

MATFORSK, Norwegian Food Research Institute, Oslovn. 1, N-1430 Ås, Norway

[1]The Norwegian School of Veterinary Science, P.O. Box 8146 Dep., 0033 Oslo, Norway

[2]Norwegian Meat Research Centre, P.O. Box 396, 0513 Oslo, Norway

Corresponding author and request for reprints: Hilde Nissen, MATFORSK, Norwegian Food Research Institute, Oslovn. 1, N-1430 Ås, Norway

Tel: +47 64970100

Fax: +47 64970333

E-mail: hilde.nissen@matforsk.no

000075

Abstract

Growth of the pathogens *Yersinia enterocolitica, Listeria monocytogenes, Escherichia coli* O157:H7 and strains of Salmonella was compared in ground beef packed in modified atmospheres of 60 % CO_2/ 40 % N_2 /0.4 % CO (high CO_2/ low CO mixture), 70 % O_2/ 30 % CO_2 (high O_2 mixture) and in chub packs. The ground beef was inoculated with rifampicin-resistant or nalidixic acid/streptomycin-resistant strains (final concentration 10^2-10^3 bacteria/g) and stored at 4 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 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. *Listeria monocytogenes* showed very little growth at 4 °C in all treatments. At 10 °C there was slow growth 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 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:k:1,5,(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. This study shows that the prolonged shelf life at 4 °C did not increase growth of *Y. enterocolitica*

and *L. mononcytogenes* in ground beef stored in the high CO_2/ low CO mixture mixture, but the observed growth of strains of salmonella at 10 °C in this mixture and in chub packs does emphasise the importance of temperature control during storage.

Keywords:

Ground beef, modified atmosphere packaging, high CO_2, carbon monoxide, *Yersinia enterocolitica, Listeria monocytogenes, Escherichia coli* O157:H7.

1. Introduction

Ground beef for retail sale is most often ready-packed in modified atmospheres (MA) or in chub packs. MA-packed ground beef has a longer microbiological shelf life and also maintains an attractive red colour. For the past decade the Norwegian meat industry has been using a gas mixture of 60-70 % CO_2, 30-40 % N_2, 0.3-0.5 % CO. (The CO comes ready mixed in the N_2 from the supplier.) The reason for adding CO to the gas mixture is that it will produce a long-lasting cherry-red colour of the meat (Sørheim et al., 1999), but the low concentration of CO has little effect on the microflora of the meat (Clark et al., 1976; Gee and Brown, 1978; Luno et al., 1998). The use of CO at such low concentrations does not present any toxic threat to the consumers (Sørheim et al., 1997). The most commonly used gas mixture for retail-ready meat in other European countries is 70 % O_2/30 % CO_2 (Gill, 1996). The high oxygen concentration is needed to keep the red colour of the meat (Lambert et al., 1991). It is therefore only possible to obtain half the CO_2 concentration used in the high CO_2/ low CO mixture. The microbiological shelf life of the high O_2 mixture will be longer than in air, but less than in the high CO_2/ low CO gas mixture (Sørheim et al., 1999).

The inclusion of CO is controversial because the stable cherry–red colour can last beyond the microbiological shelf life of the meat and thus mask spoilage (Kropf, 1980). The extended shelf life obtained by MAP may under some conditions imply increased risk of growth of pathogens (Silliker and Wolfe, 1980; Hintlian and Hotchkiss, 1986; Farber, 1991; Lamberts et al., 1991). This issue has also been discussed by the European Commission (1997).

However, even if meat packed in high CO_2/ low CO mixture acquires a stable colour, the shelf life based on odour is significantly longer in the high CO_2/ low CO

mixture only at 4 °C (Sørheim et al., 1999). At this temperature *Yersinia enterocolitica* and *Listeria monocytogenes* are considered to be the most serious pathogens in meat. At abuse temperatures (>8 °C) *Escherichia coli* O157:H7 and *Salmonella* spp. also may grow and increase the health risk to the consumers. In the present study we wanted to compare growth of these pathogens in ground beef packed in a commercial Norwegian 60 % CO_2/40 % N_2/0.4 % CO (high CO_2/low CO mixture) with growth in a high O_2 (70 % O_2/30 % CO_2) gas mixture and in ground beef in chub packs during storage at 4 and 10 °C in order to evaluate the microbiological safety of the product.

2. Materials and methods

2.1. Preparation and packaging of the ground beef

The beef carcasses were de-boned, and trimmings with 14 % fat were ground through a 4 mm plate. The batch of ground beef was divided into 500 g portions which were packaged in 0.4 % CO/ 60 % CO_2/ 40 % N_2 (high CO_2/ low CO mixture), 70 % O_2/ 30 % CO_2 (high O_2) or packed in clipped chub packs. The beef was packed at a commercial meat plant within 1 hour of grinding as described by Sørheim et al. (1999).

2.2. Bacterial cultures and growth conditions

Strains of the following pathogens were inoculated in the ground beef: *Yersinia enterocolitica* (mixture of 3 strains), *Listeria monocytogenes* (mixture of 3 strains isolated from cooked sausage, Blom et al., 1997, Nissen and Holck, 1999), *Escherichia coli* O157:H7, NCTC 1200 (National Collection of Type Cultures,

Colindale, London), non-toxic strain (resistant to 100 µg/ml nalidixic acid and 1000 µg/ml streptomycin) and *Salmonella enterica* subspecies *diarizonae* serovar 61:k:1,5,(7) (*S. enterica* 61:k:1,5,(7)), mixture of 3 strains (National Institute of Public Health, Oslo). The listeria and yersinia strains were made resistant to rifampicin by spreading 0.1 ml of overnight cultures onto agar plates of TSB medium (Oxoid, CM 129) containing 50 µg/ml rifampicin (Sigma, St.Louis, MO, USA). The growth rates of the resistant strains were practically equal to those of the parent strains when tested in TSB medium in a Bioscreen instrument (Labsystem Co., Helsinki, Finland) at the same temperature, pH and a_w (NaCl) concentrations.

In a second experiment four rifampicin-resistant salmonella strains, *S.* Typhimurium, *S.* Dublin, *S.* Enteritidis and *S. enterica* 61:k:1,5,(7) were used to inoculate the MAP- packed ground beef. The growth rates (measured as above) of the resistant strains of *S.* Enteritidis and *S. enterica* 61:k:1,5,(7) were essentially the same as the parent strains while the growth rates of *S.* Dublin and *S.* Typhimurium were slightly lower.

2.3. Inoculation and storage

After packaging the ground beef was inoculated with stationary cultures (the bacteria were cultivated overnight at 30°C and kept in the refrigerator for 1 day before use) of the different pathogenic bacteria. The stock cultures were diluted in peptone water (PW) (Bacto peptone, Difco, 1g/l; NaCl, Merck, 8.5 g/l) and the strains belonging to the same species or serovars were mixed. Fifty µl of each pathogen were inoculated with a syringe through a gas probe self-sealing tape (Toray Engineering Co. Ltd, England) into one of the corners of the MA packages. The packages thus had one pathogen inoculated in each corner. In the chub packs the

pathogens were inoculated at least 3 cm apart. Packages inoculated only with *Y. enterocolitica* and *L. monocytogenes* only were stored at 4°C and analysed after 0, 2, 5, 8 and 14 days while packages inoculated with all 4 pathogens were stored at 10 °C and analysed after 0, 2, 5 and 8 days.

In the second experiment four serovars of «*Salmonella*» were inoculated in one corner each of the package of ground beef and which was stored at 10 °C and analysed after 0, 2, 5 and 7 days. Non-inoculated packages used as controls were also stored at 10 °C.

2.4. Microbial analyses

Samples of 25 g ground beef containing the inoculated pathogens were transferred to a stomacher bag and mixed with 150 ml peptone water (8.5 g NaCl, 1.0 g peptone/1000 ml water). One hundred µl of a ten-fold dilution series were plated on blood agar containing 50 µg/ml rifampicin for *L. monocytogenes* and *Y. enterocolitica* or 100 µg/ml nalidixic acid and 1000 µg/ml streptomycin sulphate for *E. coli* O157:H7. From the undiluted mixture an aliquot of 1 ml was also plated out. For enumeration of *Salmonella* spp. the selective medium Brilliant Green Agar (modified) (BGA; Oxoid, Basingstoke, Hampshire, England) was used. The colonies were confirmed on Triple Sugar Iron Agar (TSI; Difco, Detroit, MI,) and Urea agar (Urea Agar Base, Oxoid CM53 and Urea Solution, Oxoid SR20) followed by agglutination by monovalent antisera (provided by the National Institute of Public Health). In the second experiment, samples for detection of the four salmonella strains were plated on blood agar containing 50 µg/ml rifampicin samples from non-inoculated packages were treated the same way and plated on MRS plates (CM359, Oxoid), pH 5.7, for determination of lactic acid bacteria and PCA (Difco, Detroit, MI, USA) plates for total

counts of bacteria. The plates were incubated at 30°C for up to 2 days, all aerobically. On each sampling date the packs with MA were analysed for O_2 and CO_2 and the pH for all samples was measured in the stomacher solution. Samples from two replicate packages were used for all analyses, except after 7 days storage in experiment 2 where three replicate packages were analysed.

2.5. Statistical analyses

Microbial data were subjected to analysis of variance (ANOVA) and Tukey's pairvise comparisons. It was deemed appropriate to perform ANOVA on these data after a $\log_{10}$ transformation, thereby obtaining a distribution more akin to the normal distribution on which ANOVA is based.

3. Results

As expected the shelf life of the ground beef stored at 4 °C was prolonged in the high CO_2/ low CO mixture compared with the other packaging methods. This was due to the stable colour and reduced background flora resulting in little off-odour. Thus the ground beef packed in the high CO_2/ low CO mixture still had an acceptable smell after 14 days of storage at 4 °C, while the beef packed in high O_2 mixture and in the chub packs had some off-odours. The difference in shelf life was less at 10 °C. After 5 days storage the ground beef packed in the high CO_2/ low CO mixture had an acceptable smell (except the packages inoculated with salmonella, while beef packed in the high O_2 mixture and the chub packs had a slight off-odour.

After 8 days storage there was a strong off-odour for all treatments, but the ground beef in the high CO_2/ low CO mixture still looked bright red, in accordance with Sørheim et al. (1999). The O_2 content in the high CO_2/ low CO mixture was virtually

zero throughout storage at both temperatures. At 10 °C the O_2 content in the high O_2 gas mixture decreased from 70 to about 35 % after 8 days storage, probably due to aerobic bacterial metabolism. The chub packs had an O_2-permeable casing which probably was the cause of the high bacterial growth in these packs at both temperatures.

Growth of *Y. enterocolitica* was totally inhibited both at 4 and 10 °C in the high CO_2/ low CO mixture (Fig. 1a and b), while the number in the samples packed in the high O_2 mixture increased from about $5x10^2$ cfu/g at day 0 to about 10^4 cfu/g at day 5 at 4 °C and to 10^5 cfu/g at 10°C. Growth in the chub packs at 4 °C was even higher than in the other treatments. Growth in chub packs was also higher than in high O_2 at 10 °C p=0.007). *L. monocytogens* (Fig. 2a) showed very little growth at 4 °C in all treatments. At 10 °C (Fig. 2b) there was slow growth (from about $5x10^3$ bacteria/g to about 10^4 at day 5) in the high CO_2/ low CO mixture. This was more than 10-fold higher cfu/g at day 5 than in the high O_2 mixture (p= 0.040) and the chub packs (p=0.035). Ground beef inoculated with *E. coli* O157:H7 and strains of salmonella was stored at 10°C. Growth of *E. coli* O157:H7 was slow both in the high CO_2/ low CO mixture and the high O_2 mixture (Fig. 3) and the numbers were less than 10^4 cfu/g at day 5. Growth in the chub packs was greater than in the high CO_2/ low CO-mixture (p=0.011) and in the high O_2 mixture (p=0.019), reaching 10^5 cfu/g. Growth of lactic acid bacteria in the non-inoculated packages was somewhat inhibited in the high CO_2/ low CO mixture, especially at 4 °C (Fig. 4). At start of the experiment the pH in the ground beef was about 5.8 in all packages. After 5 days storage the pH was about 5.7 in the high CO_2/ low CO mixture, 5.5 in the high O_2 mixture and 5.3 in the chub packs.

Due to growth of other bacteria on the selective plates, only approximate numbers of *S. enterica* 61:k:1,5,(7) were obtained, but growth of about 1.5 log units was observed both in the CO mixture and the chub packs (results not shown). This increase was not seen in the high O_2 mixture. To verify these results and check whether they were valid for other serovars more virulent to humans, such as *S.* Typhimurium, *S.* Dublin and *S.* Enteritidis, a second experiment was performed. The results (Fig. 5 a, b, c and d) show that after 2 days of storage at 10 °C there was essentially no growth of the salmonella strains in ground beef packed in the high CO_2/ low CO mixture and in the high O_2 mixture, while the numbers of salmonella in the chub packs were about 10 fold higher. After 5 days there was a slight off-odour in all the packages except for one package with high CO_2/ low CO mixture which smelled strongly of H_2S. In this package the numbers of all the salmonella strains were higher than in the replicate package and were of the same magnitude as the numbers in the chub packs. In the O_2 mixture there was no growth of *S.* Dublin and *S.* Enteritidis and only a low growth of *S. enterica* 61:k:1,5,(7) and *S.* Typhimurium. The growth of the salmonella strains was still greatly inhibited in the high O_2 mixture, while growth in the high CO_2/ low CO mixture was just as high or even higher than in the chub packs.

In the non-inoculated packages the lactic acid bacteria rapidly constituted most of the background flora (not shown). After 5 days storage the numbers were higher in the chub-packed samples, but after 8 days there were no obvious differences (Fig. 6). The pH in the non-inoculated ground beef followed the same pattern as in experiment 1.

4. Discussion and Conclusions

Ground beef is a high-risk product because pathogens may be mixed into the ground product which may not be sufficiently heated before consumption. To inhibit growth of spoilage bacteria and increase shelf life, MAP is often used by retailers. The question «Do modified atmospheres enhance risk to the consumers health, but delay signs of spoilage» raised by Hintlian and Hotchkiss (1986) is therefore relevant. When evaluating the safety of ground beef in the high CO_2/ low CO mixture compared to other commercially available packaging methods, we have focused on bacteria that show good growth below 10 °C and are most relevant for meat products.

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, 1999). In our study, growth of *Yersinia enterocolitica* was totally inhibited in ground beef packed in the high CO_2/ low CO mixture even at 10 °C while it grew fairly well both in the high O_2 mixture and in the chub packs. Manui-Tawiah et al. (1993) found that pork shops packed in different MA with 20 or 40 % CO_2 with or without O_2 allowed growth of *Yersinia enterocolitica*, but here the CO_2 concentration was lower than in the high CO_2/ low CO mixture (60 %) used in our study.

Listeria monocytogenes is also a pathogen that grows well at low temperatures, but in our study there was no growth of this bacterium in the ground beef in any of the packages at 4 °C, and only slow growth at 10 °C. This agrees with results of

Farber and Daley (1994) who found no growth of *L. monocytogenes* in different meat products when stored at 4 °C.

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. This is in accordance with the predictive model of Sutherland et al. (1997). Their study showed that *E. coli* O157:H7 is relatively tolerant of CO_2, but growth could be inhibited at 10 °C at high CO_2 concentrations and pH < 6.0.

In our study, growth of *Salmonella* spp. was not inhibited in ground beef packed in high CO_2/ low CO mixture and stored at 10 °C, contrary to what is found in many other studies (e.g. D'Aoust, 1991). Although salmonella may grow well and out-compete the background flora on fresh meat stored at 10 °C (Alford and Palumbo, 1969; Mackey and Kerridge, 1988), most reports claim that growth will be inhibited in MAP at this temperature (Siliker and Wolfe, 1980; D'Aoust, 1991; Gill and DeLacy, 1991). Nychas and Tasson (1996) found that high CO_2 atmospheres were more inhibitory for growth of *S.* Enteritidis on fresh poultry at 10 °C than were high O_2 atmospheres, the opposite of what we found for ground beef. Inhibition of bacterial growth may, however, be influenced by pH, texture and the composition of the product, and Gill and DeLacy (1991) did find growth of *S.* Typhimurium in high-pH beef packed in CO_2 and stored at 10 °C. Oxidative stress reactions in salmonella have recently been reported (Stephen et al., 1999). This may explain the inhibition of growth (longer lag phase) in the high O_2 mixture in our study.

The present study shows that the prolonged shelf life (due to stable colour and reduced background flora) at 4 °C did not increase the risk of growth of *Y. enterocolitica* and *L. monocytogenes* in ground beef stored in the high CO_2/ low CO

gas mixture. This is probably due to the high CO_2 concentration that is inhibitory to most microorganisms (Dixon and Kell, 1989). Even at the abusive temperature of 10 °C, the numbers of pathogens at the end of the shelf life (5 days) were less or the same as were found in the chub packs. The observed growth of salmonella in the CO mixture and chub packs does however emphasise the importance of temperature control during storage. There is a wide range of temperature criteria for chilled foods at retail in European countries. The values range from –1 °C to 10 °C, with most temperatures being between 4 and 8 °C (European Commission, 1996). These aspects should also be considered together with the conclusions of the EU report (European Commission, 1997) which state that MAP has proven to enhance the product quality by inhibiting the spoilage bacteria. MAP may also constitute a hurdle to the growth of some pathogens, and the safety of MAP products are mostly threatened by temperature abuse.

Aknowledgements We thank Oddvin Sørheim, MATFORSK, Vestfold-Buskerud-Telemark Slakteri A/L, Sem and Åse Spangelo, Hydrogas AS Utviklingssenter, Porsgrunn for assistance in the production and packing of the ground meat. We also thank Janina Berg, Birgitta Baardsen and Ingvild Rosshaug for able technical assistance and Per Lea for statistics. The Agricultural Food Research Foundation and "Omsetningsrådet" financially supported the work.

References

Alford, J.A., Palumbo, S.A. (1969) Interaction of salt, pH and temperature on the growth and survival of salmonellae in ground pork. Appl. Microbiol. 17, 528-532.

Blom, H., Nerbrink, E., Dainty, R.H., Hagtvedt, T.A., Borch, E., Nissen, H., Nesbakken, T. 1997. Addition of 2.5 % lactate and 0.25 % acetate controls growth of *Listeria monocytogenes* in vacuum packed sensory acceptable servelat sausage and cooked ham stored at 4 °C. Int. J. Food Microbiol. 38, 71-76.

Clark, D.C., Lentz, C.P., Roth, W.D. (1976) Use of carbon monoxide for extending shelf-life of prepackaged fresh beef. Can. Inst. Food Sci. Technol. J. 9, 114-117.

D'Aoust, J.Y. (1991) Psychrotrophy and foodborne *Salmonella*. Int. J. Food Microbiol. 13, 207-216.

Dixon, N.M., Kell,D.B. (1989) A review. The inhibition by CO_2 of the growth and metabolism of micro-organisms. J. Appl. Bacteriol. 67, 109-136.

European Commission (1996) Scientific co-operation on questions relating to food. Task 2.2: Studies relating to temperature control. Directorate-General III, 10.5.1996, pp. 31-33.

European Commission (1997) Food science and tecniques. Reports of the scientific committee for food (40th series) Dirctorate-General for Industry, pp. 51-62.

Farber, J.M. (1991) Microbiological aspects of modified-atmosphere packaging tecnology – a review. J. Food Protect. 54, 58-70.

Farber, J.M., Daley, E. (1994) Presence and growth of *Listeria monocytogenes* in naturally-contaminated meats. Int. J. Food Microbiol. 22, 33-42.

Gee, D.L., Brown, W.D. (1978) Extension of shelf life in refrigerated ground beef under an atmosphere containing carbon dioxide and carbon monoxide. J. Agric. Food Chem. 25, 274-276.

Gill, C.O., Delacy, K.M. (1991) Growth of *Escherichia coli* and *Salmonella typhimurium* on high-pH beef packed under vacuum or carbon dioxide. Int. J. Food Microbiol. 13, 21-30.

Gill, C.O. (1996) Extending the storage life of meats. Meat Sci. 43 (Suppl), S99-S109.

Hintlian, C.B., Hotchkiss J.H. (1986) The safety of modified atmosphere packaging: a review. Food Technol. 40, 70-76.

Kropf, D.H. (1980). Effects of retail display conditions on meat color. Proc. of the Reprocial Meat Conference, 33, 15-32.

Lambert, A.D., Smith, J.P., Dodds, K.L. (1991) Shelf life extension and microbiological safety of fresh meat – a review. Food Microbiol. 8, 267-297.

Luno, M., Beltran, J.A., Roncales, P. (1998) Shelf-life extension and colour stabilisation of beef packaged in a low O_2 atmosphere containing CO: loin steaks and ground meat. Meat Sci. 48, 75-84.

Mackey, B.M., Kerridge, A.L. (1988) The effect of incubation temperature and inoculum size on growth of salmonellae in minced beef. Int. J. Food Microbiol. 6, 57-65.

Manu-Tawiah, W., Myers, D. J., Olson, D.G., Molins, R.A. (1993) Survival and growth of *Listeria monocytogenes* and *Yersinia enterocolitica* in pork chops packaged under modified gas atmospheres. J. Food Sci. 58, 475-479.

Nesbakken, T. (1999) *Yersinia* spp. In: Lund, B., Baird-Parker, T., Gould, G. (Eds.),The microbiological safety and quality of food, in press.

Nissen, H., Holck, A.L. (1998) Survival of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella kentucky* in Norwegian fermented, dry sausage. Food Microbiol. 15, 273-279.

Nychas, G.-J.E., Tassou, C.C. (1996) Growth/survival of *Salmonella enteritidis* on fresh poultry and fish stored under vacuum or modified atmosphere. Lett. Appl. Microbiol. 23, 115-119.

Silliker, J.H., Wolfe, S.K. (1980) Microbiological safety considerations in controlled-atmosphere storage of meats. Food Technol. 34, 59-63.

Stephens, P.J., Druggan, P., Nebe-von Caron, G. The significance of oxidative stress in food preservation. Food Microbiology and food safety into the next millenium. In: Tuijtelaars, A.C.J., Samson, R.A., Rombouts, F.M., Notermans, S., (Eds.), Proceedings of the Seventeenth International Conference of the International Committee on Food Microbiology and Hygiene (ICFMH), 13-17 September 1999, Veldhoven, The Netherlands, pp. 764-766.

Sutherland, J.P., Bayliss, A.J., Braxton, D.S. Beumont, A.L. (1997) Predictive modelling of *Escherichia coli* O157:H7: Inclusion of carbon dioxide as a fourth factor in a pre-existing model. Int. J. Microbiol. 37, 113-120.

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

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

Fig. 1. Growth of *Yersinia enterocolitica* inoculated in ground beef packed in high CO_2/ low CO mixture (0.4 % CO/ 60 % CO_2/ 40 % N_2), high O_2 (70 % O_2/ 30 % CO_2) or in chub packs. The ground beef was stored at **a**, 4 °C or **b**, 10 °C.

Fig. 2. Growth of *Listeria monocytogenes* inoculated in ground beef packed in high CO_2/ low CO mixture (0.4 % CO/ 60 % CO_2/ 40 % N_2), high O_2 (70 % O_2/ 30 % CO_2) or in chub packs. The ground beef was stored at **a**, 4 °C or **b**, 10 °C.

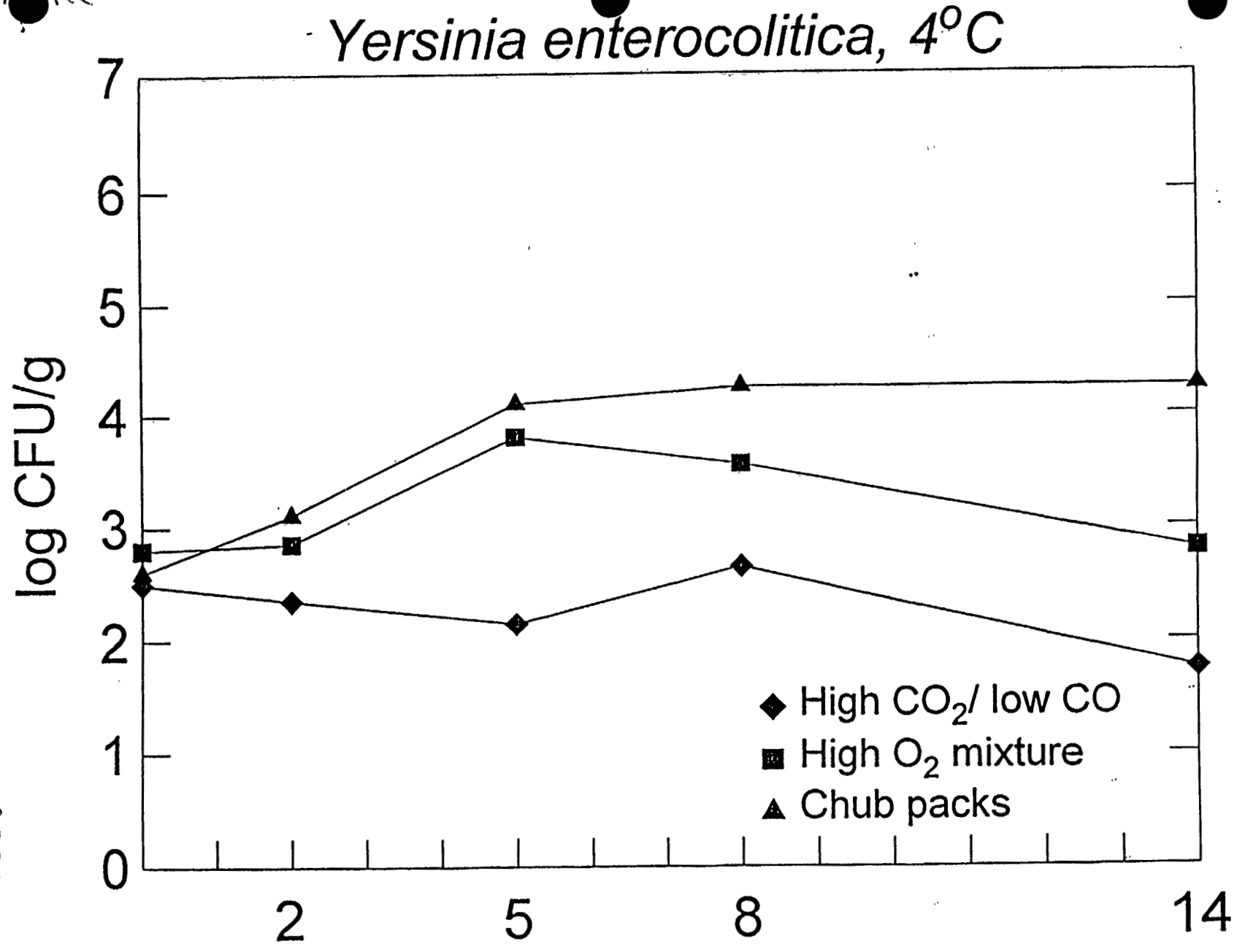
Fig. 3. Growth of *Escherichia coli* O157: H7 inoculated in ground beef packed in high CO_2/ low CO mixture (0.4 % CO/ 60 % CO_2/ 40 % N_2), high O_2 (70 % O_2/ 30 % CO_2) or in chub packs, stored at 10 °C.

Fig. 4. Growth of lactic acid bacteria (cfu/g on MRS, pH 5.7) in non-inoculated ground beef packed in high CO_2/ low CO mixture (0.4 % CO/ 60 % CO_2/ 40 % N_2), high O_2 (70 % O_2/ 30 % CO_2) or in chub packs. The ground beef was stored at **a**, 4°C or **b**, 10 °C.

Fig. 5. Growth of strains of *Salmonellae* inoculated in ground beef packed in high CO_2/ low CO mixture (0.4 % CO/ 60 % CO_2/ 40 % N_2), high O_2 (70 % O_2/ 30 % CO_2) or in chub packs, stored 10 °C. **a.** *S.*Typhimurium **b.** *S.* Dublin **c.** *S.* Enteritidis **d.** *S. enterica* 61:k:1,5,(7).

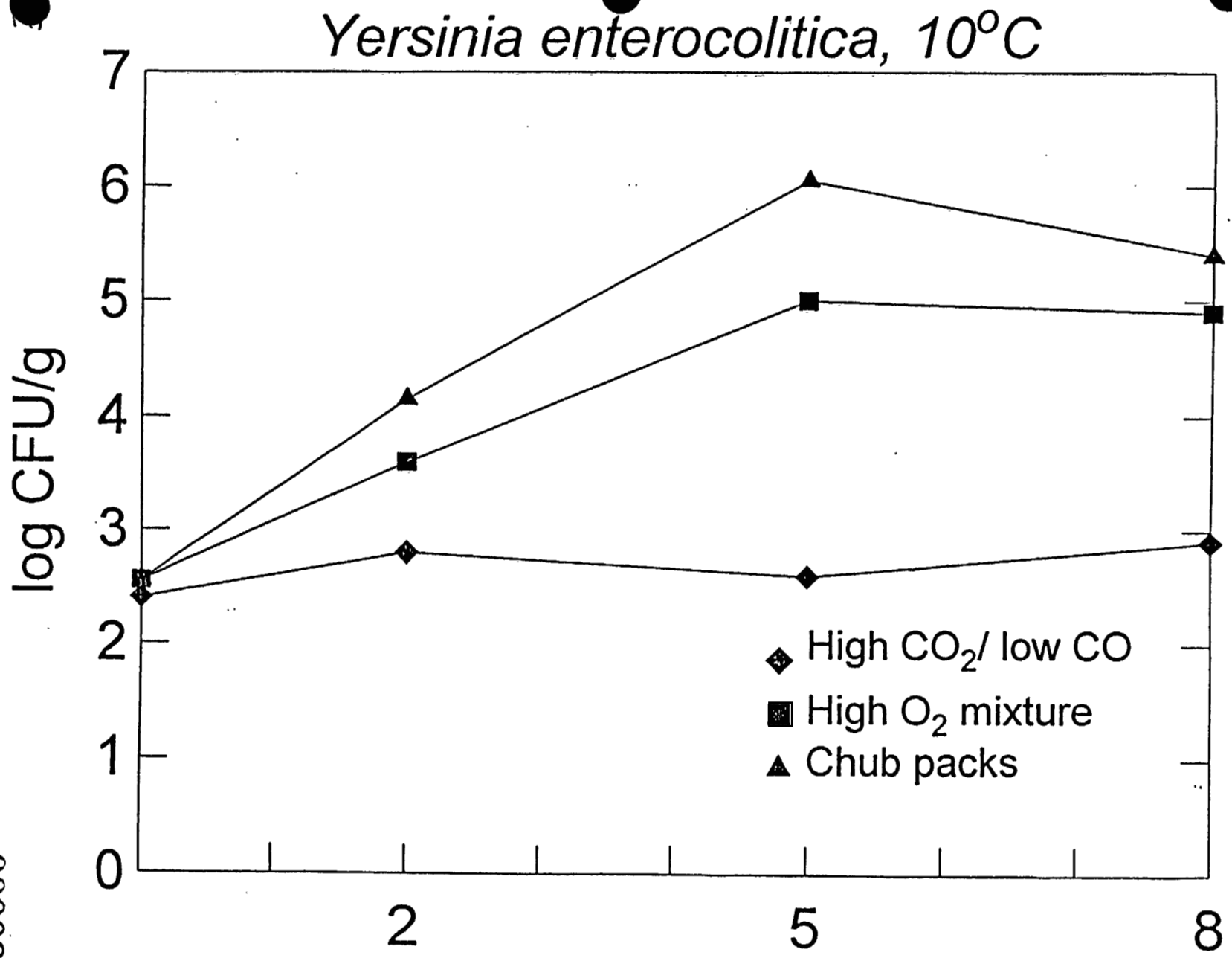
Fig. 6. Growth of lactic acid bacteria (cfu/g on MRS, pH 5.7) in non-inoculated ground beef packed in high CO_2/ low CO mixture (0.4 % CO/ 60 % CO_2/ 40 % N_2), high O_2

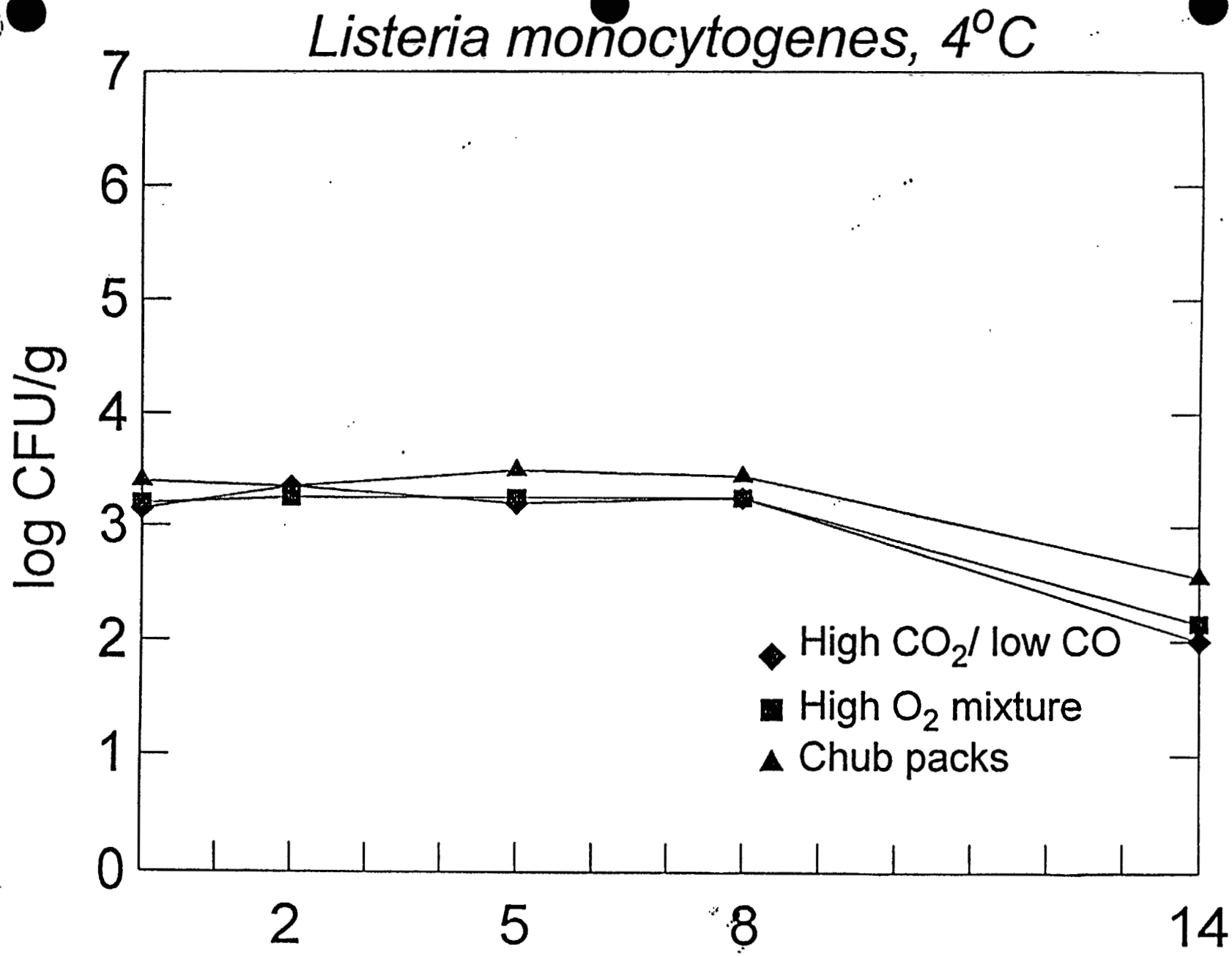
(70 % O_2/ 30 % CO_2) or in chub packs. The ground beef was stored at **a**, 4 °C or **b**, 10 °C.



000094

000094

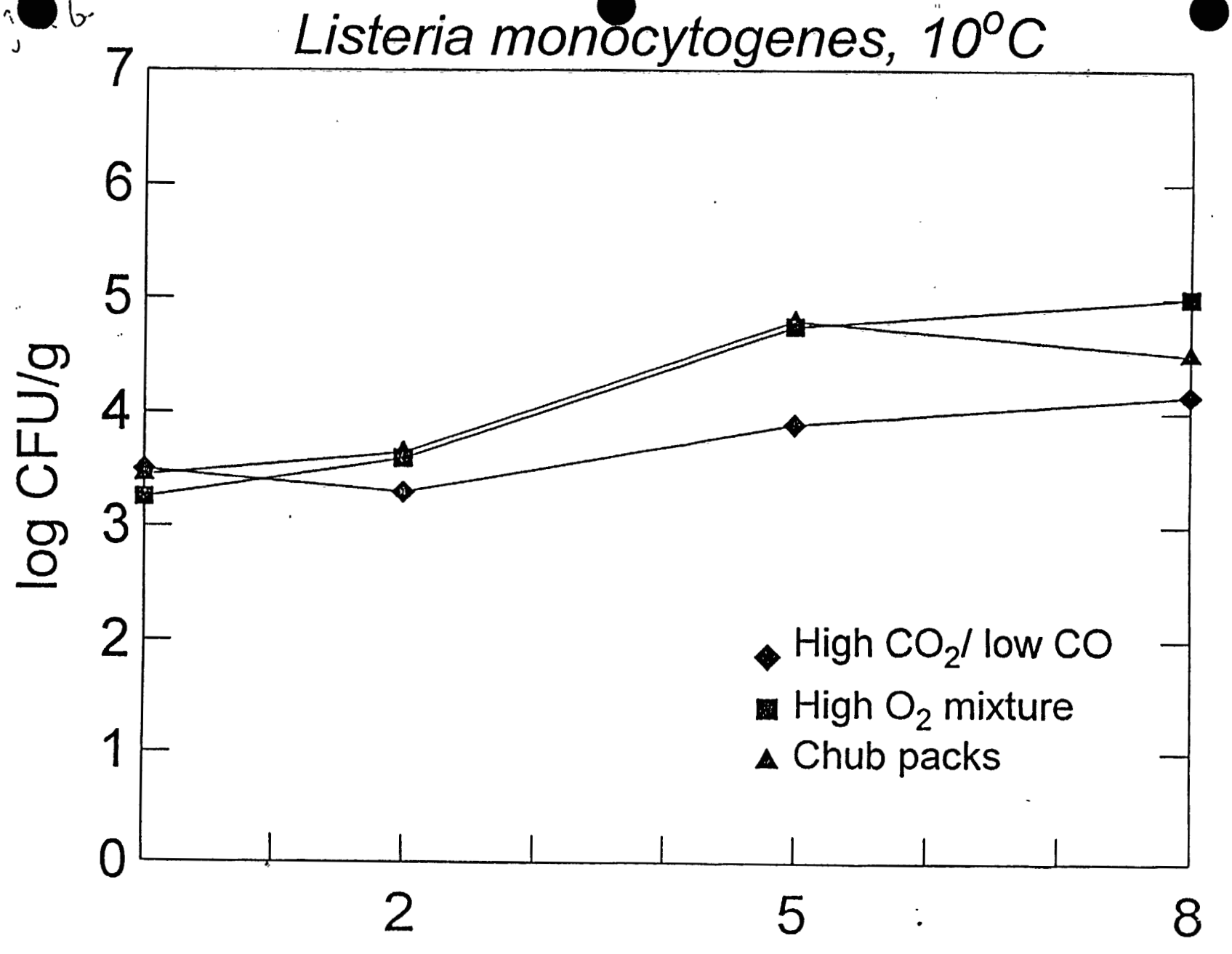

Yersinia enterocolitica, 10°C
log CFU/g
0
1
2
3
4
5
6
7
2
5
8
High CO_2/ low CO
High O_2 mixture
Chub packs



960000

960000

Fig. 6



000097

000097