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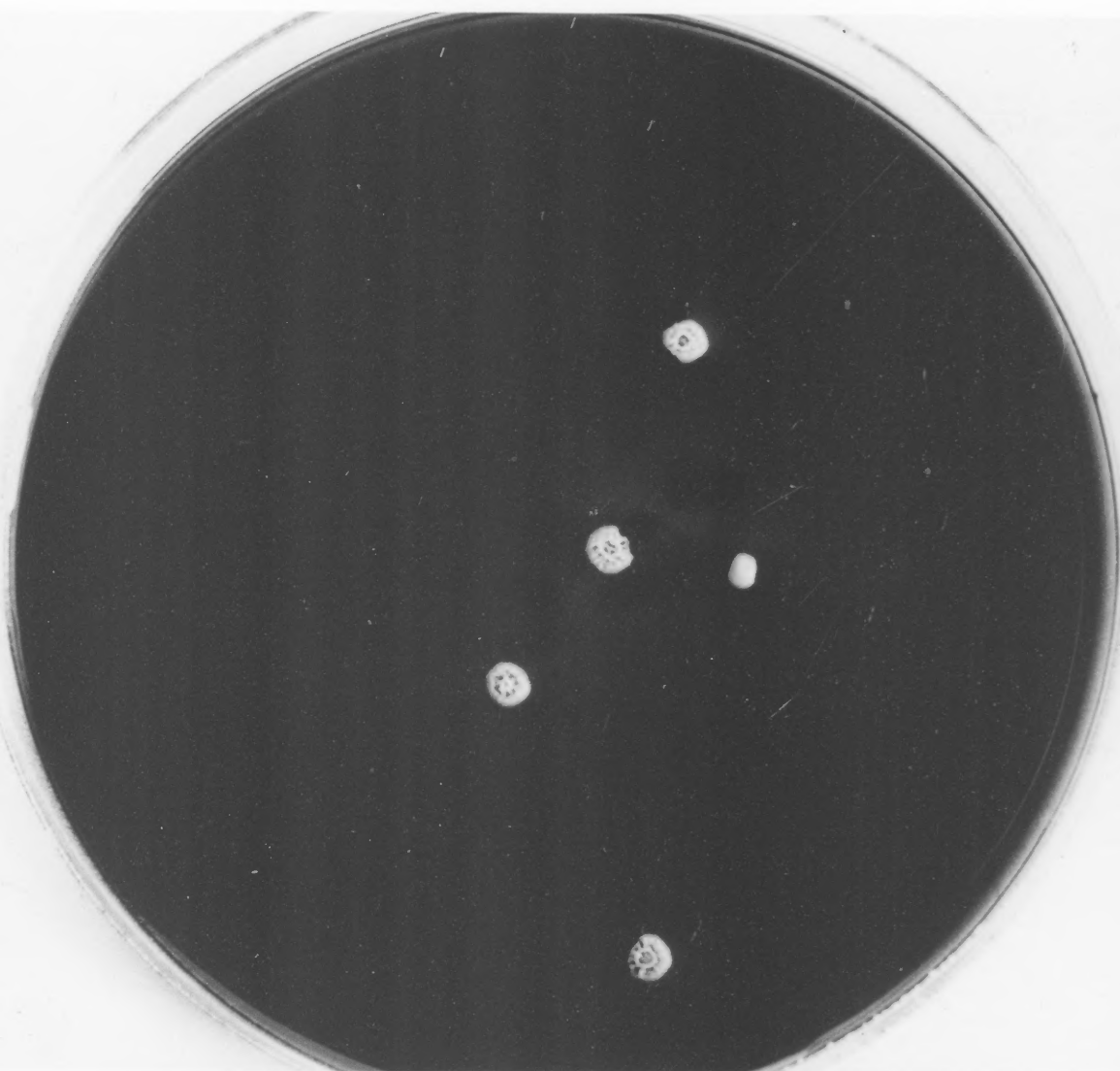
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DAIRY, FOOD AND ENVIRONMENTAL

# SANITATION

OCTOBER 1994



A Publication of the International Association of Milk, Food and Environmental Sanitarians, Inc.

*"The mission of IAMFES is to provide food safety professionals worldwide with a forum to exchange information on protecting the food supply"*



# This is Your Personal Invitation to Join

The International Association of Milk, Food and Environmental Sanitarians, founded in 1911, is a non-profit educational association of food protection professionals. The IAMFES is dedicated to the education and service of its members, specifically, as well as industry personnel in general. Through membership in the Association, IAMFES members are able to keep informed of the latest scientific, technical and practical developments in food protection. IAMFES provides its members with an information network and forum for professional improvement through its two scientific journals, educational annual meeting and interaction with other food safety professionals.

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The Association is comprised of a diverse membership of over 3,500 from 38 nations. IAMFES members belong to all facets of the food protection arena. The main groups of Association members fall into three categories: Industry Personnel, Government Officials and Academia.

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**Dairy, Food and Environmental Sanitation** — Published monthly, this is the official journal of IAMFES. Its purpose is the disseminating of current information of interest to the general IAMFES membership. Each issue contains three to five informational applied research or general interest articles, industry news and events, association news, columns on food safety and environmental hazards to health, a food and dairy industry related products section, and a calendar of upcoming meetings, seminars and workshops. All regular IAMFES members receive this publication as part of their membership.

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**Editor's Note:** *Included in this issue of Dairy, Food and Environmental Sanitation, is the Call for Papers for the 82nd IAMFES Annual Meeting, and the Announcement of the IAMFES Developing Scientists Awards. Please turn to the Green Section inserted in the middle of this issue for details.*

**ABOUT THE COVER . . .** *New Bacto Candida Isolation Agar allows users to rapidly identify Candida albicans with a high degree of accuracy and predictability. Photo courtesy of Difco Laboratories, Inc., Detroit, MI.*

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**Membership Dues:** Membership in the Association is available to individuals only. Dues are \$60 per year and include a subscription to Dairy, Food and Environmental Sanitation. Dues including both Dairy, Food and Environmental Sanitation and Journal of Food Protection are \$90.00. Student membership is \$30.00 per year, with verification of student status, and includes Dairy, Food and Environmental

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# Thoughts From the President . . .



By  
C. Dee Clingman  
IAMFES President

## Controversy is an Opportunity for Education

In today's world we are surrounded by experts. Experts, experts, experts, and more experts. Specialization in a professional discipline is the "rule-of-thumb" these days. When was the last time you went to a general practice physician? Attorneys now specialize in corporate law, tax law, employment law, insurance litigation, criminal law, civil law, etc. I recently called my attorney to help out a business associate who had a son who was arrested for a traffic violation, and my attorney replied, "I don't handle criminals." Well, excuse me.

It is amazing that as more people become specialists the more difficult it is to get quick, accurate and responsive information. Maybe there was some serenity in the statement "take two aspirins and call me in the morning if you are still ill." At least it was quick, responsive to the need, and directed actions for correction. Today, an ill person would be referred to another specialist, who would refer him to someone else who would give him a CAT scan, who would refer him to someone else to interpret the results and on and on... Maybe the two aspirins would have done it for a 37 cent investment. As our "advanced society" becomes more specialized, we are becoming less efficient. Recently, when traveling outside of the U.S., I was talking to a food protection specialist in another country about *Escherichia coli*. He knew a wealth of information about the organism, its impact and controls as they relate to *his country*. Yet he was not a specialist (microbiologist) practicing a specialty (food microbiology). He probably didn't know the current research that demonstrated *E. coli* growth 20,000 leagues under the sea, on a full moon, on the second Tuesday of the month... Have we become too specialized that we cannot apply the knowledge we have? Or communicate it to others? Have we become the CAT scan expert that can't interpret the results?

By now you are thinking, what does this column have to do with the title? Think about it...

As we move and process more information by computer, we have less time to examine it. We become so consumed with data collection, data generation, data entry and data analysis, that no one sees the forest for the trees. But do we ever know about those trees? As environmental health and food safety professionals, we must be careful that the data does not consume us. We must challenge ourselves to be more aware of the periphery of the "specialty" of our choice. We must understand the human needs and be able to explain our findings and recommendations to those less knowledgeable about the area.

Controversy is an opportunity for education. If there are no conflicts or challenges to our lives we become complacent. If your boss says to you, "this report is not what I would have expected of you to produce," you bet your adrenaline will kick in and that report will be rewritten with a different attitude. The act of questioning what we do and what others do is not in itself negative, it is the education process, perhaps educating the person asking the question or causing the respondent to question themselves. All in all, education will occur.

Remember, absence of evidence is not evidence of absence.

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- Is the largest and most broad-based scientific food trade association.
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- Participated in the HACCP Round Table and HACCP Round Table Steering Committee of the U.S. Department of Agriculture.
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# On My Mind . . .



By  
*Steven K. Halstead, CAE*  
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*Executive Manager*

is sanitation....

I am asking you to bear with me on this. I openly admit that I am going to be right on the edge of how much I know about this topic and I may well go over the edge. If I do, you will know it before I do, so I ask that you cut me some slack and not get too wrapped up in the details.

I am concerned that too often SANITATION is synonymous with CLEANUP or MAINTENANCE. Instead of receiving recognition as being one of the most important jobs in a food processing operation, it is too often looked upon as the least desirable, if not, the most hated job in the plant.

Maybe it goes back to when we were children and Mom yelled at us about cleaning up our rooms, or from our first family job of washing the dishes. Or our first "real" job was in a restaurant washing dishes or busing tables. Whatever the case, SANITATION is not receiving the recognition it deserves.

A couple of years ago, my older son, Robert, spent the summer on a sanitation crew in a meat packing plant. He didn't learn a whole lot about sanitation, but he learned a great deal about foreign languages. (He was the only English speaking employee on the crew.) It seems that because of the low esteem held for the position and perhaps the low pay, only immigrants (and college students) will take the positions.

One aspect of the job that really bothered Robert was the lack of sensitivity of management to the cultural differences of the employees. While Robert didn't know much about sanitation, he did have an appreciation for the need for

cleanliness. He did have knowledge of bacteria and microorganisms and the damage they can do.

Robert tried to talk to his colleagues about food poisoning, but didn't get too far. His friends could understand the concept of sickness, but they had absolutely no concept of the causes of illness. Microbes were simply beyond their comprehension. "How could something you can't even see, hurt you?" they would ask.

Robert tells me that he was never instructed as to why he was doing what he was doing, only how to do it. Any instruction he did receive was in English, meaning that it was of virtually no value to the rest of the crew. In three months, he was never sure when he had done the job properly except when the "inspector" made him do it over.

He did receive quite a bit of instruction in the use of safety equipment, i.e., hearing protection, gloves, boots, hard hat, etc. He was using live steam containing some kind of chemical. He was never informed as to what the sanitizer was, but it was strong enough to bleach the skin on his arms in an eight hour shift. Seeing the priority given to worker safety, one could only conclude that worker compensation cases were more numerous than food poisoning cases.

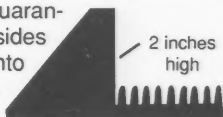
And yet, the plant's entire reputation relied on the ability of that crew to sanitize the work area. One slip up and hundreds, if not thousands of people could become sick and the company totally wiped out.

I wonder if those who set the wages ever think about that?

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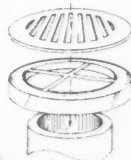
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# Food Safety Issues: Press Reports Heighten Consumer Awareness of Microbiological Safety

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## ABSTRACT

Recent food scares have shaken consumers' confidence in the microbiological safety of their food and water supply. A search of newspaper articles published in nine national newspapers from January 1, 1990 through December 1993 found 206 articles pertaining to the microbiological safety of the food and water supply (Table 1). In 1990, and again in 1991, most articles focused on the presence of *Salmonella* in eggs and egg products. *Vibrio cholera* was the microorganism most frequently cited in newspaper articles published during 1992. Contamination of the water supply by the protozoan parasite *Cryptosporidium* and contamination of food, specifically ground beef, by the bacterium *Escherichia coli* O157:H7 were responsible for the majority of articles published in 1993. From January 1, 1990 through December 1993, two Wisconsin newspapers published 98 articles pertaining to the microbiological safety of food and water. Pathogens cited most frequently were *Salmonella* in 1990, the hepatitis A virus in 1991, *Listeria* in 1992 and *Cryptosporidium* in 1993. With the widespread publicity given these and other pathogens, consumers are becoming more aware of the microbiological safety of the food and water supply. The hazard analysis critical control point system (HACCP) and certification of foodservice managers are two approaches that can be used to

allay consumer's fears and prevent the spread of foodborne disease in the United States.

Although most foodborne disease outbreaks reported to the Centers for Disease Control and Prevention (CDC) between 1973 and 1987 were caused by microorganisms (94-97, 99-105), consumers have traditionally been more concerned with chemical (food additives and pesticides) rather than microbiological safety (246). Recent food scares involving *Escherichia coli* O157:H7 in hamburger (46,53,54,58,59,69, 89,109,116,121,125,126,144,183,203,210), *Salmonella enteridis* in eggs (41,124,145,150,167,178,182,224,230,243, 244), outbreaks of hepatitis A caused by infected foodhandlers (19, 33, 34, 52, 62, 65,152,172,173) and contamination of drinking water by the parasite, *Cryptosporidium*, (4, 5, 55,67, 68,138,139,146,160,176, 217, 232, 233) have shaken consumer confidence in the microbiological safety of the nation's food and water supply. With the widespread publicity given to these and other food and water related incidents, consumers appear more concerned than before about the microbiological safety of their food and water (Table 1).

Factors that contribute to outbreaks of foodborne disease are identified in this review. Practices that commonly contribute to outbreaks of foodborne disease as well as preventive measures are discussed.

TABLE 1. Newspaper articles pertaining to the microbiological safety of the food and water supply (Jan. 1990 through Dec. 1993).\*

DATE	ARTICLE	SOURCE
Dec. 23, 1993	A mystery on every plate	New York Times (122)
Dec. 10, 1993	For cities, a big battle against microorganisms	Washington Post (163)
Dec. 8, 1993	100 become ill after Md. party	Washington Post (215)
Nov. 25, 1993	Fannin hepatitis outbreak may be linked to trailer park	Atlanta Journal and Atlanta Constitution (134)
Oct. 26, 1993	Parasite found again in Milwaukee water	New York Times (55)
Oct. 23, 1993	Bad meat	Washington Post (45)
Oct. 14, 1993	Meat industry buckles down to tackle foodborne illness	Chicago Tribune (111)
Oct. 14, 1993	Water contamination problem kept quiet in Arlington	Washington Post (108)
Oct. 12, 1993	Claims of bad water cause bad blood in Pennsylvania	Wall Street Journal (73)
Oct. 8, 1993	Four in Waycross recovering from suspected botulism	Atlanta Constitution (51)

DATE	ARTICLE	SOURCE
Oct. 7, 1993	An unpleasant birthday surprise: 30 partygoers, guest of honor included, get food poisoning	Washington Post (74)
Oct. 5, 1993	Sunday dinner puts Gumbel on sideline	USA Today (162)
Oct. 1, 1993	D.C. ordered to urge boiling of tap water	Washington Post (107)
Sept. 30, 1993	Area of NE told to boil its water	Washington Post (106)
Sept. 30, 1993	Tainted food made managers sick during DWP strike	Los Angeles Times (154)
Sept. 17, 1993	<i>Salmonella</i> fells wedding guests	Washington Post (71)
Aug. 21, 1993	Illness traced to tainted shark	Los Angeles Times (119)
Aug. 20, 1993	Search gains in Oregon <i>E. coli</i> outbreak	New York Times (57)
Aug. 15, 1993	Red meat becoming rare treat	Chicago Tribune (205)
Aug. 13, 1993	Doctors say a new cholera poses a worldwide danger	New York Times (2)
Aug. 7, 1993	Bacteria rate is still high in New York City tap water	New York Times (237)
Aug. 5, 1993	Coping with bacteria in water	New York Times (239)
Aug. 3, 1993	Poisoning of two kids tied to raw burgers	Chicago Tribune (210)
Aug. 1, 1993	Don't worry but be wary: A fine line in water scare	New York Times (240)
Jul. 31, 1993	Water crisis is extended in Chelsea	New York Times (241)
Jul. 29, 1993	Bacterial taint in water supply baffles experts	New York Times (238)
Jul. 29, 1993	Water alert	New York Times (66)
Jul. 28, 1993	Bacteria in water prompts warnings in Manhattan	New York Times (156)
Jul. 25, 1993	Mountain club moves to clean up its own act	Boston Globe (82)
Jul. 24, 1993	State recalls raw milk tainted with <i>Salmonella</i>	Los Angeles Times (61)
Jul. 19, 1993	Sick cooks can infect diners, CDC warns	Atlanta Constitution (152)
Jul. 4, 1993	Health agency to track illness caused by parasite	New York Times (176)
Jul. 1, 1993	Last patient is released in Jack-in-the-Box case	New York Times (54)
Jun. 22, 1993	Customers at Syracuse, NY, Taco Bell showing symptoms of food poisoning	Wall Street Journal (48)
Jun. 6, 1993	<i>Salmonella</i> victims rise to 49	Chicago Tribune (72)
Jun. 4, 1993	Melrose Park restaurant linked to <i>Salmonella</i>	Chicago Tribune (148)
May 28, 1993	30 slaughterhouses shut down in surprise USDA inspections	Washington Post (229)
May 22, 1993	Central State's kitchen 'atrocious,' inspector says	Atlanta Journal and Atlanta Constitution (110)
May 20, 1993	Polls show doubts on food safety	Los Angeles Times (200)
May 19, 1993	Killers in the food supply	Atlanta Constitution (235)
May 15, 1993	High bacteria levels found in Bay Ridge water	New York Times (77)
May 11, 1993	Contaminated cider sparks inquiry	Washington Post (168)
May 5, 1993	Cider can harbor dangerous bacteria	USA Today (159)
Apr. 29, 1993	Cholera flares in Mexico City	Los Angeles Times (112)
Apr. 28, 1993	Springtime pulls water, sodas amid fears of contamination	Chicago Tribune (247)
Apr. 25, 1993	Water parasite linked to six Milwaukee deaths	New York Times (67)
Apr. 20, 1993	Science Times: Outbreak of disease in Milwaukee undercuts confidence in water	New York Times (4)
Apr. 19, 1993	After 9 deaths in Florida, oyster industry confronts raw fear	Washington Post (81)
Apr. 19, 1993	Milwaukee eyes ways to protect its water	Chicago Tribune (232)
Apr. 12, 1993	'Life goes on' as Milwaukee battles parasite-laced water	USA Today (146)
Apr. 12, 1993	Worrying about Milwaukee's water	Chicago Tribune (68)
Apr. 10, 1993	Officials, residents in Milwaukee struggle with outbreak of illness	Los Angeles Times (217)
Apr. 10, 1993	Study hinted at a parasite problem in Milwaukee	New York Times (139)
Apr. 9, 1993	Illness brews in Milwaukee water supply	USA Today (160)
Apr. 9, 1993	Milwaukee's bad water a gut-wrenching issue	Chicago Tribune (233)
Apr. 9, 1993	Milwaukee's water suspected as cause of intestinal illness	New York Times (138)
Apr. 9, 1993	Uncommon parasite tied to ills in Milwaukee	New York Times (5)
Apr. 6, 1993	Who's watching the hen-house? Outfoxing the bacteria that cause <i>Salmonella</i> in eggs	Washington Post (230)
Apr. 4, 1993	Bacteria outbreak no shock	Boston Globe (121)

TABLE 1. *Continued.*

DATE	ARTICLE	SOURCE
Apr. 1, 1993	\$1 million settlement in food poisoning suit	Los Angeles Times (199)
Mar. 30, 1993	Sizzler hit by food poisoning outbreak	Los Angeles Times (90)
Mar. 28, 1993	Second outbreak of bacterial infection is reported	New York Times (58)
Mar. 22, 1993	Word in <i>E. coli</i> case in RI is prevention, not overreaction	Boston Globe (70)
Mar. 19, 1993	Rare-meat bacteria strikes girl in RI	Boston Globe (69)
Mar. 18, 1993	<i>Salmonella</i> controlled, officials say	Chicago Tribune (234)
Mar. 4, 1993	The bacteria next time	Los Angeles Times (201)
Mar. 3, 1993	Agriculture Dept. policy blamed for tainted food	New York Times (93)
Feb. 28, 1993	Illness that killed 63 in France is traced to pork	New York Times (208)
Feb. 23, 1993	CDC narrows search for source of contaminated burger patties	Los Angeles Times (89)
Feb. 23, 1993	Investigators nearing source of bacteria that killed three	New York Times (53)
Feb. 22, 1993	Meat is deadly	USA Today (144)
Feb. 22, 1993	17-month-old is 3rd child to die of illness linked to tainted meat	New York Times (59)
Feb. 18, 1993	Meat inspection: An accident waiting	Washington Post (6)
Feb. 17, 1993	Tainted burgers trigger new safety efforts	Boston Globe (183)
Feb. 12, 1993	Health: Tainted-hamburger incident heats up debate over U.S. meat inspection system	Wall Street Journal (125)
Feb. 11, 1993	Meat inspection: 'No longer adequate'	Los Angeles Times (198)
Feb. 9, 1993	Science times: Lessons are sought in outbreak of illness from tainted meat	New York Times (3)
Feb. 5, 1993	Espy expected to urge tougher testing following tainted-hamburger deaths	Wall Street Journal (242)
Feb. 5, 1993	USDA meat inspection system under fire	Los Angeles Times (123)
Jan. 31, 1993	Secondary cases from bacteria worry Northwest	Boston Globe (204)
Jan. 30, 1993	Tainted meat linked to California cattle	Los Angeles Times (63)
Jan. 29, 1993	Burger 'horror' spreads fear	USA Today (126)
Jan. 29, 1993	Poisoning by bacteria kills second child	Los Angeles Times (109)
Jan. 28, 1993	Clean up burger act	USA Today (47)
Jan. 27, 1993	Tainted hamburger raises doubts on meat safety	New York Times (116)
Jan. 25, 1993	State says chain involved in outbreak didn't comply with new cooking rule	Wall Street Journal (140)
Jan. 23, 1993	Boy dies in bacterial outbreak tied to hamburger chain in West	New York Times (46)
Jan. 23, 1993	Restaurant not to blame for tainted meat, officials say	Los Angeles Times (129)
Jan. 22, 1993	Firms reject blame in food poisoning	Los Angeles Times (203)
Jan. 12, 1993	How safe is airline food?	Washington Post (79)
Jan. 7, 1993	30 inaugural workers taken ill after lunch	Washington Post (64)
Jan. 1, 1993	Hepatitis outbreak feared in Denver area	New York Times (52)
Dec. 17, 1992	33 students are hospitalized after Brooklyn school lunch	New York Times (207)
Dec. 9, 1992	Just how safe is the food we eat?	Atlanta Constitution (225)
Dec. 9, 1992	More viruses thrive in food supply, doctors say	USA Today (218)
Dec. 8, 1992	Was it something you ate? Disagreeable symptoms are usually not food allergies	Washington Post (202)
Nov. 24, 1992	Healthy Choice cheese loaf is recalled from markets	Wall Street Journal (32)
Nov. 17, 1992	Science Times: Sewage bacteria in food chain	New York Times (231)
Nov. 1, 1992	<i>Salmonella</i> poisoning strikes 145 who attended luncheon	New York Times (132)
Sept. 12, 1992	Care urged after <i>Salmonella</i> death	Washington Post (136)
Sept. 11, 1992	Number of cholera cases reaches a 31-year high	Atlanta Constitution (142)
Sept. 11, 1992	U.S. cholera cases in '92 outnumber other years	Wall Street Journal (42)
Sept. 11, 1992	U.S. cholera cases set record in 1992	New York Times (43)
Aug. 19, 1992	RI health officials to probe cause of illness among scouts	Boston Globe (39)
Aug. 16, 1992	Tainted water forces hardship on Pawtucket, RI	New York Times (40)
Aug. 4, 1992	Bacteria taint water in RI city	Boston Globe (114)
Jul. 23, 1992	Unsafe seafood: The risks are high	Los Angeles Times (197)



DATE	ARTICLE	SOURCE
Jul. 14, 1992	15 at casino treated for food poisoning	New York Times (31)
Jun. 15, 1992	Amtrak to improve food service in wake of findings by FDA	Wall Street Journal (27)
May 14, 1992	Cholera infected 102 on aircraft	Washington Post (28)
Apr. 16, 1992	Scientists urge precautions against rare but often fatal food poisoning	Washington Post (227)
Apr. 15, 1992	Moms-to-be should avoid soft cheeses	Atlanta Constitution (214)
Mar. 18, 1992	Food company accused of selling tainted items	New York Times (167)
Mar. 13, 1992	Cholera reaching gulf ports	Atlanta Constitution (213)
Mar. 7, 1992	U.S. poultry, meat supply to be tested	Atlanta Journal and Atlanta Constitution (88)
Feb. 27, 1992	FDA inspection finds 20% of seafood tainted	Los Angeles Times (196)
Feb. 22, 1992	Illness toll in cholera scare soars	Los Angeles Times (157)
Feb. 22, 1992	Outbreak of cholera on flight affects 65	Boston Globe (120)
Feb. 21, 1992	Cholera alert after death of air traveler	USA Today (130)
Feb. 21, 1992	Cholera kills one and fells many on flight	New York Times (174)
Feb. 21, 1992	Cholera strikes six on Argentine flight	Washington Post (29)
Feb. 21, 1992	25 on flight to LAX show signs of cholera	Los Angeles Times (158)
Feb. 20, 1992	Effort made to contact people on plane in which five passengers suffered cholera	Los Angeles Times (137)
Feb. 17, 1992	Flaim's medal failure tough to stomach: Food poisoning ails U.S. speed-skater	Chicago Tribune (135)
Feb. 9, 1992	Hamburger hazard: Deaths traced to bacteria in ground beef raise questions about meat inspections and cooking techniques	Washington Post (165)
Feb. 9, 1992	Oscar Mayer orders recall of hot dogs	Chicago Tribune (37)
Feb. 9, 1992	U.S. meat inspections come under scrutiny	Washington Post (228)
Jan. 25, 1992	Poultry officials vow to cut contaminants	Atlanta Journal and Atlanta Constitution (186)
Jan. 21, 1992	Parasite, rare in U.S., reported on both coasts	Washington Post (38)
Jan. 19, 1992	Moynihan falls ill with signs of food poisoning	New York Times (141)
Jan. 16, 1992	Study of retail fish markets inds. wide contamination and mislabeling	New York Times (92)
Jan. 14, 1992	To dismay of Caesar salad fans, New Jersey bans the runny egg	Boston Globe (41)
Dec. 28, 1991	FDA warns against eating organs of Dungeness crab	Los Angeles Times (17)
Nov. 20, 1991	Health alert at restaurant	New York Times (19)
Oct. 15, 1991	Make sure fish isn't foul	USA Today (20)
Sept. 21, 1991	Three Medical cases of cholera reported	Washington Post (128)
Sept. 15, 1991	Food poisoning reported in CT	Boston Globe (18)
Sept. 5, 1991	Shellfish warning sought	Los Angeles Times (195)
Sept. 2, 1991	Need to boil water persists in New Bedford	Boston Globe (155)
Sept. 1, 1991	USDA chief takes action on food-safety "problem"	Atlanta Journal and Constitution (87)
Aug. 26, 1991	Cholera's mysterious journey north	Washington Post (80)
Aug. 25, 1991	Cantaloupe scare has area farmers melancholy over sales	Washington Post (219)
Aug. 22, 1991	State's growers blame cantaloupe losses on 'scare' by U.S. agencies	Los Angeles Times (171)
Aug. 19, 1991	Tainted dairy foods part of bigger problem	Atlanta Constitution (23)
Aug. 16, 1991	Officials trace outbreak of <i>Salmonella</i> poisoning to cantaloupes	New York Times (21)
Aug. 16, 1991	Cantaloupe identified as culprit in national <i>Salmonella</i> outbreak	Washington Post (14)
Aug. 15, 1991	Cut cantaloupes pose hazard	Los Angeles Times (191)
Aug. 15, 1991	U.S. report says butter, cheese plants unsanitary	Boston Globe (24)
Aug. 15, 1991	U.S. warns of contaminated dairy products	Los Angeles Times (25)
Jul. 11, 1991	New outcry over poultry bacteria	Los Angeles Times (194)
Jul. 11, 1991	The market: Chicken has replaced pork as dinner-time risk because of threat of <i>Salmonella</i>	Chicago Tribune (189)
Jun. 30, 1991	Stop poultry plants from breaking rules	Atlanta Journal and Atlanta Constitution (22)
Jun. 29, 1991	Consumer warning urged on chicken	Atlanta Journal and Atlanta Constitution (84)
Jun. 28, 1991	Chicken industry must clean up its act	USA Today (170)
Jun. 12, 1991	The summer spoilers: Beware the bacteria	Boston Globe (187)

TABLE 1. *Continued.*

DATE	ARTICLE	SOURCE
May 31, 1991	Poisoned meat from Canada	New York Times (113)
May 29, 1991	Bush's doctor orders test of water supply	Washington Post (180)
May 26, 1991	A day to forget: "Most sick I've ever been"	Atlanta Journal and Atlanta Constitution (83)
May 26, 1991	High-tech plants churn out birds — but add to risk	Atlanta Journal and Atlanta Constitution (85)
May 26, 1991	Millions of tainted birds make it to market, inspectors say	Atlanta Journal and Atlanta Constitution (86)
May 26, 1991	Unsafe chicken betrays consumer	Atlanta Journal and Atlanta Constitution (26)
May 23, 1991	Even with warnings, safety isn't guaranteed	USA Today (16)
May 17, 1991	Five more cases of hepatitis A are reported in Sauk Village	Chicago Tribune (117)
May 16, 1991	How safe is your food?	Chicago Tribune (206)
May 3, 1991	Fresh eggs in hot water at restaurants	Wall Street Journal (124)
May 1, 1991	Kids checked in attempt to trace source of hepatitis A outbreak	Chicago Tribune (118)
Apr. 30, 1991	Hepatitis A outbreak baffles Sauk Village	Chicago Tribune (216)
Apr. 24, 1991	New Jersey cholera cases tied to illegal food import	Los Angeles Times (193)
Mar. 14, 1991	Like its cousin <i>Salmonella</i> , 'campy' is a major player in diarrhea	Chicago Tribune (188)
Mar. 7, 1991	Legal beat: Food poisoning	Wall Street Journal (131)
Feb. 28, 1991	Concern voiced on uncooked pork	New York Times (15)
Feb. 21, 1991	How safe is our seafood?	Los Angeles Times (192)
Feb. 20, 1991	Contaminants may pose a risk to otherwise healthful fish	USA Today (127)
Jan. 30, 1991	<i>Salmonella</i> outbreaks traced to eggs	Boston Globe (150)
Jan. 9, 1991	More fish for dinner — and more worries, too	Atlanta Constitution (179)
Nov. 16, 1990	Faster slaughter lines are contaminating much U.S. poultry	Wall Street Journal (143)
Nov. 12, 1990	300 Colorado students fall ill; food suspected	Boston Globe (12)
Oct. 30, 1990	Update on the state of poultry may spur readers to chicken out	Atlanta Constitution (236)
Oct. 23, 1990	<i>Salmonella</i> : Nothing to crow about	Atlanta Constitution (212)
Oct. 17, 1990	Holes in the fish safety net	New York Times (9)
Oct. 17, 1990	On the trail of <i>Salmonella</i>	Washington Post (226)
Oct. 11, 1990	1,008 reported food poisoning symptoms, firm says	Chicago Tribune (244)
Oct. 7, 1990	Raw batter can contain bacteria	Boston Globe (145)
Oct. 6, 1990	Conventioneers' illness probe pointing to eggs	Chicago Tribune (243)
Oct. 5, 1990	Investigators trying to pinpoint source of conventioneers' illness	Chicago Tribune (223)
Oct. 4, 1990	Convention illness linked to <i>Salmonella</i>	Chicago Tribune (222)
Oct. 3, 1990	City hunts for source of convention illness	Chicago Tribune (221)
Oct. 2, 1990	Stricken conventioneers taken to area hospitals	Chicago Tribune (153)
Sept. 15, 1990	Fresh eggs linked to food poisoning	Atlanta Journal and Atlanta Constitution (178)
Aug. 23, 1990	Raw turkey harbors harmful bacteria	Los Angeles Times (190)
Aug. 8, 1990	Tomatoes connected to <i>Salmonella</i> cases in four Midwest States	New York Times (13)
Jul. 11, 1990	Battling an elusive bacterium	Los Angeles Times (209)
Jun. 4, 1990	Sandwich maker defends plant	Washington Post (10)
Jun. 1, 1990	Stein and health chief fight over safety of takeout food	New York Times (91)
May 23, 1990	Drury Lane shuts down its kitchen	Chicago Tribune (184)
May 22, 1990	Fish inspections needed to improve city	Atlanta Constitution (8)
May 8, 1990	The bacteria at the table	New York Times (11)
May 5, 1990	Hepatitis outbreak tied to supermarket	Chicago Tribune (149)
Apr. 9, 1990	Tainted well keeps water tight	Chicago Tribune (166)
Apr. 5, 1990	Chitlins good for the soul — but not always the body	Atlanta Constitution (185)
Mar. 20, 1990	Eat defensively to avoid foodborne disease	Washington Post (211)
Feb. 23, 1990	Inspectors find contaminated cheese at plant	Atlanta Constitution (76)
Feb. 5, 1990	Inspection system can't keep up with fast-paced poultry processing	Chicago Tribune (151)
Jan. 24, 1990	Questions raised on role of cheese in <i>Salmonella</i> infections	New York Times (1)
Jan. 16, 1990	Undercooked eggs linked to food poisoning	Atlanta Constitution (224)
Jan. 5, 1990	Food poisoning from eggs spreads	USA Today (182)

\*The source for articles was Newspaper Abstracts on Disc: Computer file - Ann Arbor, Michigan. University Microfilms 1993-1990.

#### *Factors that contribute to outbreaks of foodborne illness.*

With the consolidation of small slaughter and food processing operations into larger units (147), today's consumers are eating more mass produced foods (49). More American women are also working outside of the home (175). Therefore, Americans have less time to spend preparing meals and are consuming more partially prepared foods and fast foods (49). The foodservice industry, noted for its high turnover rates and lack of on the job training programs, is further characterized by workers who have no concept of personal hygiene (161). Individuals with chronic illnesses are living longer while the number of people infected with the human immunodeficiency virus is increasing (115). Both populations are highly susceptible to infectious diseases (49).

These factors can all contribute to outbreaks of foodborne illness. Many factors are also responsible for the emergence of "new" foodborne pathogens, such as *Listeria monocytogenes*, and *E. coli* O157:H7. When these "new" pathogens were implicated in large outbreaks of foodborne illness (46, 53, 54, 59, 63, 89, 109, 121, 126, 140, 144, 208), or found in the food or water supply (30, 35, 36, 44, 60, 76, 159, 168, 238) they attracted the attention of the press.

#### *Practices that contribute to foodborne disease outbreaks.*

According to CDC Surveillance Summaries from 1973 through 1987 (94-97, 99-105), mishandling of food in foodservice establishments accounted for 44.6% of the total outbreaks reported, while food prepared in the home was responsible for 20.5% of reported outbreaks. Practices thought to contribute to foodborne disease were reported for 3,720 outbreaks. Improper holding temperature was associated with most of the outbreaks for all reporting years. The remaining four factors, in order of frequency of occurrence, were poor personal hygiene, inadequate cooking, contaminated equipment and obtaining food from unsafe sources. Multiple factors usually contributed to single outbreaks.

#### *Improper holding temperature.*

In June of 1983, complaints of severe gastroenteritis were reported by 71% of 260 guests following an outdoor barbecue at the Defense Language Institute located in Monterey, California. *Salmonella infantitidis* was isolated from chicken and from 84% of the ill individuals. On the morning of the barbecue, chicken was parboiled by the morning cooks and then placed in a large container and refrigerated. Later in the day, evening cooks, assuming that the chicken had been adequately cooked, covered the chicken with barbecue sauce and warmed it over a charcoal fire. Insufficient cooking and improper holding were two factors that contributed to this outbreak of salmonellosis (177). Poultry products have to be cooked to an internal temperature >74°C (2165°F) and then placed in shallow containers for storage in the refrigerator.

#### *Poor personal hygiene.*

An outbreak of gastroenteritis caused by the Norwalk virus occurred in 1982 in the Minneapolis/St. Paul area. This outbreak of viral gastroenteritis (5,000 cases) was caused by an infected bakery worker who stirred a vat full of buttercream frosting with his bare hand and arm (7). Frosting was used to cover pastries and the finished products were then distributed

throughout the area. The bakery worker became ill on his way to work but claimed he washed his hands between bouts of diarrhea (133). Infected individuals should never handle foods while ill.

#### *Inadequate cooking.*

Since mid-January 1993, *E. coli* O157:H7 has infected over 500 individuals in Washington, Idaho, California and Nevada (50); three deaths have been reported (53). The cause of this outbreak was traced to the consumption of undercooked hamburger served at Jack-in-the-Box restaurants (56). Although the Washington State Health Department set a minimum internal temperature of 68°C (155°F) for cooking hamburgers, company policy required that hamburgers only be cooked to an internal temperature of 60°C (140°F). This temperature is not sufficient to kill *E. coli* O157:H7. The parent company claimed it was unaware of the new temperature requirement (169).

As in most outbreaks, multiple factors contributed to this outbreak of foodborne disease. Some lots of frozen hamburger patties shipped to the west coast Jack-in-the-Box restaurants were contaminated with *E. coli* O157:H7. Samples taken from one production run found >2.4 x 10<sup>2</sup> *E. coli* O157:H7/g of meat (78). Regulatory agencies and industry groups now recommend that foodservice operators cook ground meat to an internal temperature of 68°C (155°F) (169).

#### *Contaminated equipment.*

During the last two weeks of December 1980, five cases of salmonellosis were reported in communities located near Milwaukee, WI. All of the ill individuals had attended a pre-Christmas party for employees at a Milwaukee hotel. The Milwaukee Health Department identified an additional 36 individuals with stool cultures positive for *Salmonella enteritidis*. An inspection of the hotel's kitchen revealed numerous errors in food preparation and handling (181). Inadequate sanitation of cutting boards and cutting instruments was one of the errors cited. Foodservice equipment needs to be washed, rinsed and sanitized between every use.

#### *Obtaining food from unsafe sources.*

Forty-six cases of botulism occurred in persons who had eaten hot sauce in a Mexican restaurant in Pontiac, MI. No deaths were reported. All ill persons had eaten hot sauce either by adding it to their food or eating nachos which contained the sauce. The hot sauce was prepared with red tomato sauce and home-canned jalapeno peppers. A sample of the home-canned peppers and stools from ill persons were found to contain type B botulinum toxin (98). Serving home-canned foods in foodservice establishments is a violation of state health regulations.

#### *Preventive measures.*

Changes in agricultural practices, a growing population susceptible to infectious diseases, lifestyle changes, the emergence of "new" foodborne pathogens and the high turnover rate reported for workers in the foodservice industry indicate that new approaches are needed to allay consumers' fears and prevent the spread of foodborne disease in the United States.

The hazard analysis critical control point (HACCP) system and certification of foodservice managers are two such approaches.

Originally developed by the Pillsbury Company, the National Aeronautics and Space Administration and the U.S. Army, Natick Laboratories, HACCP is a preventive program for quality control that attempts to bring potential dangers to the attention of management. Hazard analysis has been defined as the identification of sensitive ingredients, critical processing points and human factors that affect product safety. Critical control points have been described as processing determiners whose loss of control would result in an unacceptable food safety risk (75). HACCP has slowly emerged as the primary approach to assuring the microbiological safety of the food supply (164).

Certification of foodservice managers may also improve the sanitation practices of foodservice establishments. Results of a 1989 survey regarding certification of foodservice managers found that three states had mandatory (7) certification programs, 17 states had voluntary certification programs and 20 states had local jurisdictions with certification programs (220). The state of Wisconsin has recently moved in the direction of mandatory certification of foodservice managers. By January 1, 1995, all restaurant managers and/or operators in Wisconsin must pass a course on basic restaurant sanitation (245).

The HACCP system and certification of foodservice managers are two approaches that can be used to reduce the incidence of foodborne disease attributed to foodservice establishments throughout the 1990s and into the next century. These approaches are interrelated since foodservice managers knowledgeable in all aspects of food safety are the key to effective HACCP programs.

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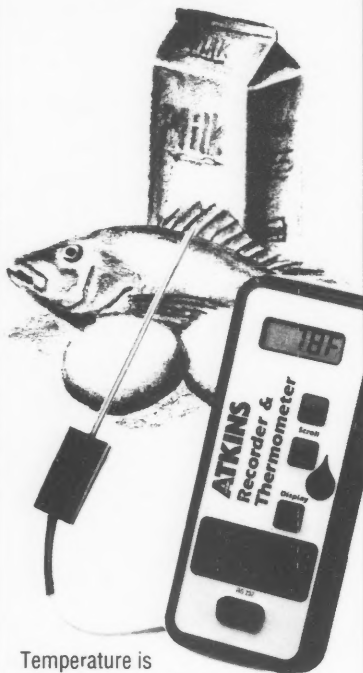
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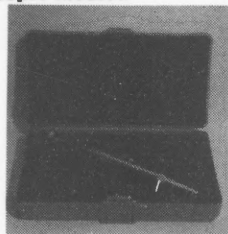
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# Foodborne Illness (Part 12)

## Shigellosis

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*Shigella* bacteria (four species) are the cause of shigellosis, a diarrheal illness; it is commonly known as bacillary dysentery. This agent is associated with the intestinal tract of humans (also primates) and is rarely found in other animals. The usual source of the organisms is asymptomatic carriers; convalescent patients may also be carriers; shigellae may persist for months in the intestinal tract and be shed in feces. *S. sonnei* is responsible for about 80% of cases in the U.S.

The bacterium is a gram-negative facultative rod which does not form a spore. All four species elaborate a toxin that has enterotoxic, neurotoxic, and cytotoxic activities, responsible for the inflammatory responses in the intestinal areas; a bacteremia rarely occurs. Shigellae are generally not hardy and do not resist environmental stresses very well. Most strains are killed at 145.4°F, 63°C in 5 min. Their temperature range for growth is 45°F, 7°C to 115°F, 46°C, with the optimum at 98.6°F, 37°C. The pH range for growth is about 5-8. Shigellae may grow well in foods with few microbial competitors. Studies have shown that shigellae may survive in a number of low-acid food products at temperatures of <77°F, <25°C for more than 50 days.

*Shigella* is a highly infectious organism and it is thought that ingestion of very few (10 to 100) of these bacteria can cause illness. Fecal-oral transmission from a patient or carrier is involved. Those individuals, especially food workers, who fail to thoroughly and effectively wash their hands and clean under fingernails after defecation may indirectly contaminate foods or water and/or directly contaminate others by physical contact. Water and milk may act as a vehicle as a result of direct fecal contamination. The incubation period can range from 12 to 96 h (usually 1 to 3 days) up to 7 days. Illness generally persists from a few days to two weeks, with most cases being self-limited. Symptoms can range from a mild diarrhea with no fever to a severe form (frequent diarrhea, containing blood and mucus [severe fluid loss], fever, and abdominal cramps). Infants and young children can be severely affected and high fevers may cause convulsions. Since transmission of this illness is most often person-to-person, it may be justified to treat with antibiotics all persons who might be considered a public health hazard (e.g., food workers and children).

Salads are most often implicated in foodborne shigellosis, with about two-thirds of the reported outbreaks associated with their consumption; salads made with potatoes, tuna,

shrimp and chicken have been particularly involved. Other foods that have been involved in outbreaks include lettuce, watermelon, cream puffs, beans and spaghetti. Outbreaks are related to foods that have been served raw or handled after cooking by *Shigella*-infected foodservice workers, often in combination with unsatisfactory refrigeration (cooling) methods.

### CONTROL/PREVENTION

- Educational sessions/handouts, with emphasis on good personal hygiene, especially thorough and effective handwashing (clean nails with a brush), is the best preventive measure; the handwashing is essential due to the low infective dose of *Shigella*. Management must provide suitable handwashing facilities, with appropriate supplies. This approach can be frustrating because of the high turnover in foodservice personnel; a large percentage of these persons stay on the job <1 year.
- Food workers with the illness or having contact with a patient must be excluded from handling foods in accord with the State Isolation and Quarantine Requirements, which call for negative stool test(s) before return to work.
- Human feces must be disposed of in a sanitary manner and not used as a fertilizer on any crops (vegetables) used as food.
- Shellfish come from approved waters and are obtained from certified suppliers; prepared and handled properly by the food worker for the consumer.
- Field workers who harvest vegetables and fruits need to be educated to wash their hands after using the toilet; under field conditions stress the use of sufficient toilet tissue to minimize hand contamination. Protect/treat water supplies used for field workers.

Food workers who practice poor personal hygiene (especially failing to wash hands thoroughly [including the use of a nail brush]) after using a restroom and then prepare (handle) foods, are the main contributing factor to outbreaks of shigellosis.

The four species of shigellae:

- *S. sonnei*
- *S. flexneri*
- *S. boydii*
- *S. dysenteriae* (usually most serious)

# HAZCON-Based Total Quality Management

## Regulatory Inspection HACCP Versus Food Operation Self-Control HACCP — Part 1

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### INTRODUCTION

The "buzzword" today is **Hazard Analysis and Critical Control Points**. It is being "sold" by the government as a "magic silver bullet" that will eliminate foodborne disease and injury in the United States. However, this government Hazard Analysis and Critical Control Points system is quite inadequate. If the weaknesses are not recognized and corrected, it will not reduce the disease and injury problem, and it will be thrown out in a few years as another "failed magic bullet."

The purpose of this paper is to highlight these deficiencies so that the food industry can focus on a correct process for identifying hazards and specifying adequate controls in order to achieve zero liability costs. Note, the food industry's goal is **zero liability costs**, not inspections, inspection scores, number of cases of persons with disease and injury (due to physical objects in food), etc. These are government objectives.

One fundamental problem is that government food inspection agencies want us to believe that government inspection assures safety. The reason is that no one in the government will ever want to reduce his/her workforce, even if inspectors are no longer needed. However, the fact is that HACCP, when implemented properly, does eliminate the need for most government inspectors, nationally and locally. The reason is simple. HACCP is process pre-control by management, and process control during operation by the line employee who fries the hamburger, slaughters the pig, cooks the stew, or serves the customer. Every minute that the process is in operation, it must be controlled if the zero defect goal is to be achieved. For it to be controlled effectively, management must have identified all hazards (i.e., possible liability costs) and taught the employee effective control procedures. The only duties of government under these conditions are to do long-range food safety research and to specify minimum food process safety standards for future processing methods.

### THE HAZARD PROBLEM

The sad fact is that food which is grown and harvested throughout the United States is grossly contaminated by fecal material from animals in the environment (e.g., forest animals, rodents, cats, birds, toxic marine plankton, etc.) and by

waste chemical by-products from industrial processes. The food is also contaminated during careless packaging and handling. Microorganisms are also allowed to multiply during distribution due to careless storage practices. Finally, it is contaminated simply by the fecal material of the people who handle the food from the point of growing and harvesting until final consumption. These problems are basic and will not disappear soon or forever. Since punishment is not an effective method for achieving performance by habit, inspection by the government is not the way to assure zero liability costs. Only positive education and coaching by a supervisor will be effective in achieving performance by habit. Hence, there is no choice but to use HACCP-based TQM self-control.

The government, as the advocate of consumer safety, is technically responsible to our society for correct hazard identification and then, the specification of minimum process safety control standards. Actually, government personnel get much of this information from research scientists in universities, colleges and industry. A clear historical example of this is the standard for the inactivation of *Clostridium botulinum* in canned food. This information was generated by the highly recognized industry experts Drs. Ball, Estes, and Meyer in the 1920s. The reason we have excellent control over our canning processes today is also the result of the outstanding work of the National Food Processors Association, which has completed most of the process control studies to identify the correct procedures for operating retorts and other food sterilization devices. Then, the universities' food science departments run the Better Process Control course to train retort operators. The government contribution has been to make it mandatory for the retort operator to know how to run the retort correctly by writing it into codes.

As a scientist involved in establishing food processing standards and procedures, I would like to point out the problems today with our current government HACCP concept as put forth by The National Advisory Committee on Microbiological Criteria for Foods (NACMCF). Then, I will suggest an improved approach, which incorporates conventional manufacturing quality control. (For examples, see 1,3.) The concepts of process control have been well understood for almost seventy years. Dr. Shewart in the late 1920s began to lay out the principles for understanding process variability

and how to keep process variability under control. This is really the foundation of HACCP. Today, the American Society for Quality Control, the American Institute for Chemical Engineers, and many other professional organizations are training managers to institute simple systems analysis and control procedures to effect major improvements in the safety of products. Before the food industry invents something new, it is prudent to examine what others have worked on for many years and has been shown to be very effective.

Another reason why industry must take charge of the HACCP process, especially in FDA-regulated processes, is that the FDA's primary responsibility is auditing and enforcing. Inspectors are told to cite code violations, not to suggest ways to solve problems because then, they become responsible if their ideas do not work. In addition, there is no evidence that there is any FDA research in retail food systems to determine correct minimum safety process control parameters. Standards are written and published for retail food operations without operational testing to show that these standards work. The result is impractical, inaccurate food process safety standards based, at best, on outdated research by food technologists that do not control today's real hazards and the causes of liability costs.

The USDA, on the other hand, has extensive research programs whereby critical limits for process standards that will assure the safety of the food are ascertained. Inherently, though, even the USDA puts forth the position that control is in the hands of its inspectors in the plants, and chastises operators with letters, criticizing the operators' behavior when a mistake is found. This "enforcement" philosophy is even more strongly implemented by state and local officials. At the state and local levels, there is very inadequate research to identify hazards and hazard levels, and more particularly, to specify correct minimal process control limits for hazard control. These government defects will be very difficult to correct because of political constraints. Meanwhile, the hazards in the food must be controlled. The solution is industry self-control.

The lack of an effective integrated government program cannot stop the industry from using HACCP. The real pay-off for industry self-control HACCP is that operators will be able to strive for zero liability costs without being encumbered by regulations with outdated information. The cost savings from the reduction in government costs to the industry provides a new opportunity for the industry, educators, and research and development specialists, and accelerates development in terms of food systems quality improvement and TQM. This leads to the highly valuable national goal of world class food excellence for which the food industry in the U.S. strives. Because of the efforts of the food industry leaders who seek this goal, the U.S. will be known worldwide as having the safest, best quality wholesale food, food markets and restaurants.

**Analysis of the Government's Seven Principles of HACCP:** the Problem in 1992, NACMCF published the document, **Hazard Analysis and Critical Control Point System (4)**. The first problem with this document is that HACCP is not a system. It is a process used by management

in a continuous quality improvement food system to assure that pathogenic substances are controlled to a safe level. A food system is defined by three components: its **inputs, processes and outputs**. A food system consists of eight functional components necessary to take input and convert it to output:

1. Management
2. Consumer
3. Employees
4. Environment
5. Facilities
6. Equipment
7. Supplies
8. Products

In developing the processes that produce the products in this food system, the company must not only consider competitive customer satisfaction and value, but must also consider the safety and liability of each item. There are three components of quality:

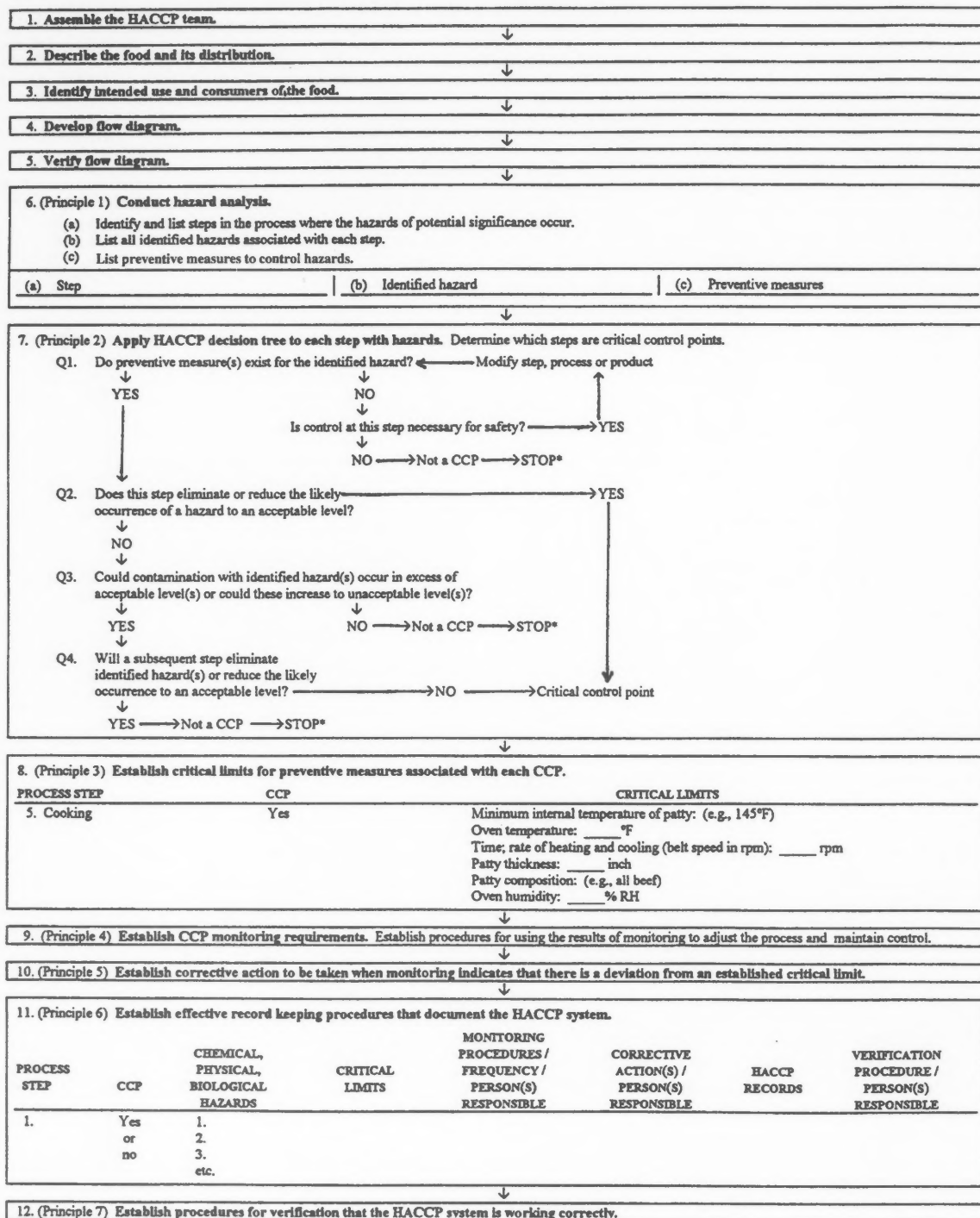
1. Product safety, zero liability costs
2. Competitive value excellence whereby consumers choose this product first among competitive choices
3. Cost effective company operation or minimum cost for the competitive quality

HACCP is simply the process whereby after a product that meets competitive customer value and needs and wants criteria is developed, management can then institute hazard control policies, procedures and standards at steps in the process which, when followed, make the company capable of achieving zero liability costs. Therefore, HACCP is part of the TQM program, and the seven NACMCF principles are simply the hazard control steps in the TQM program.

#### **PROBLEMS WITH THE NACMCF CONCEPT**

The NACMCF concept (Figure 1) is flawed in the following ways. First, it does not identify as a principle, the need for management commitment and leadership, specification of the hazard team, describing the food and its distribution, identifying the use and consumer of the food, and developing flow diagrams. These requirements are listed, but because they are not identified as a principle, they tend to be overlooked when the government explains HACCP. Actually, they are likely to be the steps in the process that are most prone to failure, that is, inadequate performance. For example, only management leadership and resources can assure HACCP success. Many programs are a one-person effort, while everyone in the company must be involved for the program to be a success. In the retail sector, distribution of the food includes the "doggie bag" that customers take home. This food is hazardous through the time of consumption because of the surviving spores. Currently, there is no control of this problem. Flow diagrams can take many forms, not just block diagrams as shown in current literature. For the retail food operation, the "flow diagram" is the recipe.

Figure 1. NACMCF Logic Sequence for the Application of HACCP.\*



\* Reference: NACMCF. 1992. Hazard analysis and critical control point system. USDA-FSIS. Washington, D.C.

### Principle No. 1

Principle No. 1 is **CONDUCT A HAZARD ANALYSIS**. The document lists three components of this principle:

1. Identify and list steps in the process where the hazard of potential significance occur
2. List all identified hazards associated with each step
3. List preventive measures to control hazards

The problem is that in the U.S., all hazards have not yet been identified by the government. For more than fifteen years, HACCP was promoted as a microbiological control process. Only recently have chemicals and physical hazards been included. The reason is simple. HACCP was begun by government employees and food microbiologists, not company managers who were paying the liability costs.

The next problem is that the U.S. government has no standards that determine safe and unsafe levels of hazards, even though the government states that HACCP allows for acceptable and unacceptable risks. There is no "zero" in microbiology, chemistry, or physical contamination. Because the NACMCF program does not define how to calculate risk, it is currently impossible to specify a preventive measure to control a hazard because no preventive measure is perfect and no safe level is specified. The government has not stated, for instance, whether the objective is to reduce *Salmonella*  $10^{-7}$  or  $10^{-3}$  in raw cooked or washed retail products. The government also must first state what level of *Salmonella* typically is on the food. Only then can one determine the effectiveness of various processes in reducing *Salmonella* to a safe level. Then, the processor can determine adequate standards and procedures for the control of *Salmonella*.

While this is simple to state, it is difficult for the government to implement because the safer the process, the less fresh and more processed is the food. Consumers over the years have wanted fresh food.

### Principle No. 2

Principle No. 2, **IDENTIFY THE CCPS IN THE PROCESS**, says to apply the HACCP decision tree to each step with hazards. The objective is to determine which steps are the true hazard control steps. This principle is dangerous. Actually, home canning exactly applies this principle. There is no required training or licensing for anyone who home cans food. The assumption is that those who can food at home will learn to do so safely either by reading equipment instructions or from friends. Since the control of *C. botulinum* is highly specific, and essentially no one goes to school to learn how to home can food safely, the critical control point as passed down from one generation to another is that all home canned food, before serving, should be brought to a boil for at least 10 minutes. The purpose is to inactivate the *C. botulinum* toxin, which can be produced in home canned food stored for as short a time as a few days. This particular application of HACCP represents our government's dangerous thinking, that it is acceptable to use a final process step to make food safe. Telling consumers to reheat leftover food to 165°F is the same type of mistake. This will not inactivate *C. botulinum* or *Staphylococcus aureus* toxins. It is a very poor control because it is ineffective in many food serving situations.

### Principle No. 3

Principle No. 3 states to **ESTABLISH CRITICAL LIMITS FOR PREVENTIVE MEASURES ASSOCIATED WITH EACH IDENTIFIED CCP**. This is technically impossible to accomplish today because of the government's failure to specify under Principle No. 1, both the level of the pathogenic substance in the product that must be reduced to an acceptable level and the acceptable level itself.

The current regulatory process control standards vary from grossly unsafe, as in the case of no specification as to how to wash fecal material from raw vegetables, to excessively safe in terms of reducing *Salmonella* on roast beef by  $10^{-7}$  per gram, when in fact there are only approximately 0.23 MPN per cm<sup>2</sup> on the surface of raw beef product (5).

### Principle No. 4

Principle No. 4, **ESTABLISH CCP MONITORING REQUIREMENTS, and ESTABLISH PROCEDURES FOR USING THE RESULTS OF MONITORING TO ADJUST THE PROCESS TO MAINTAIN CONTROL** is a correct step. Unfortunately, though, the government has not provided correct information about monitoring. This is true especially in the case of retail food whereby the 1976 FDA Food Code, which all state regulatory agencies still use, states that operators can use a bimetallic stem thermometer to measure food temperatures. The bimetallic stem thermometer is a grossly inaccurate device when used to measure small volumes of food such as hamburger, chicken breast, fish, etc. It is impossible with this type of instrument to judge pasteurization, which should be measured to  $\pm 1^\circ\text{F}$ . This government mistake is part of the root cause of the hamburger *Escherichia coli* problem today.

Also, the procedures for using the results of monitoring are not specified. It is well understood in Statistical Process Control how and when to adjust a process to achieve ultimate stability. For example, as long as the process variation is  $< 1\sigma$  and the control rules are not violated, the process operating settings such as grill temperature and time should not be changed.

### Principle No. 5

Principle No. 5, **ESTABLISH CORRECTIVE ACTION TO BE TAKEN WHEN MONITORING INDICATES THAT THERE IS A DEVIATION FROM AN ESTABLISHED CRITICAL LIMIT**, is again correct, except that there is no government guidance regarding what determines adequate corrective action. For example, if an employee loses his/her Band-Aid in a ten-gallon mixing bowl of tunafish salad, but the injury was clean and there was no *Staphylococcus* present, and the employee finds and removes the Band-Aid, can the tunafish salad be sold or must it be discarded? This and similar questions are not trivial in the retail food sector. It does happen. HACCP would indicate that the tunafish salad can be sold.

To be continued in the November 1994 issue of *Dairy, Food and Environmental Sanitation*.

# Abstracts of Papers Presented at the 81st Annual Meeting of IAMFES

San Antonio, Texas — July 30 - August 3, 1994

Abstracts of most papers submitted for presentation at the 81st Annual Meeting of the IAMFES appear on this and the following pages. The complete text of some of the papers will appear in future issues of the *Journal of Food Protection and Dairy, Food and Environmental Sanitation*.

## QUANTITATIVE RISK ASSESSMENT IN FOOD MICROBIOLOGY

### OVERVIEW - THE INTERNATIONAL COMMISSION ON MICROBIOLOGICAL SPECIFICATIONS FOR FOODS (ICMSF) APPROACH

Terry A. Roberts, Chairman ICMSF and Head, Microbiology Department, Institute of Food Research, Reading Laboratory, Barley Gate, Reading RG6 2EF UK

The philosophy in the second ICMSF book *Sampling for Microbiological Analysis: Principles and Specific Applications* is proving a useful starting point for developing concepts of foodborne microbiological risks. Account is taken of relevant properties of the microbes including the severity of the illness and the capacity for the disease to spread, and the conditions under which the food is expected to be stored, handled and consumed. This was developed into control *via* sampling rather than prevention by designed quantitative risk assessment. Challenge tests are still relied upon, but their relevance to the microbial response in foods, where the numbers of pathogens present may be orders of magnitude lower than the inoculum used, is in urgent need of clarification. In some cases, the case of growth in the challenge test equates poorly with the infrequency of illness associated with the challenge organism and the food, suggesting that our margins of safety may be larger than necessary. Models of microbial responses and summations of historical data can also be helpful adjuncts in judging risk.

### RISK ASSESSMENT TERMS AND DEFINITIONS

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Quantitative risk assessment evolved as a formal decision-aiding process for managing the uncertainties involved in determining the magnitude of risk of carcinogenic hazards. During the evolutionary process, appropriate sources of data, mechanisms for handling data gaps, and working definitions of terms have been established. The increasing desire to apply the quantitative risk assessment paradigm to infectious foodborne hazards carries with it the requirement to revisit these decisions and to modify them to accommodate differences between toxic and infectious disease processes and the availability of data. As we do this, it is important to use standardized approaches and definitions to assess risk of infectious foodborne hazards along the entire food chain to facilitate development of farm-to-table food safety programs.

### HEALTH RISK ANALYSIS OF FOOD IN CANADA

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Quantitative risk assessments are increasingly required for policy direction and resource allocation, as well as determining types of research and surveillance projects within Health Canada. Data bases to help determine these should include information on foodborne disease surveillance, laboratory isolations of enteric pathogens, high-risk populations, food consumption patterns, food preparation practices, microbial levels in specific foods, and infectious doses relative to specific foods. However, either these do not exist, are not in an adequate format to be easily used, or are questionable because they are not peer-reviewed. Nevertheless, attempts at risk assessments are being made for specific tasks and improvements will be made later as more information is generated. This is acceptable as long as the uncertainties are stated. For example, such assessments are currently being prepared to help determine the labeling requirements for raw meat and poultry, the hazards

associated with fish and shellfish, the control of cracked eggs, and the extent of the problem of raw milk consumption. Once these have been established, management options can be specified with cost benefit analyses for each option, and the recommended option stated. It is highly desirable that once an option has been chosen, an effective risk communication strategy be developed for each recommendation.

### PROCESS RELIABILITY AND RISK — A FOOD INDUSTRY PERSPECTIVE

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This paper will discuss how the food industry considers microbial risks and how it takes this into account in designing food processes and products. The elements of design important to the safety of a processed food include: the material processed; the processing conditions themselves; the packaging system, distribution and handling; and the "in-home" practices of the consumer.

Advances in microbial modelling allow us to predict accurately the likely growth, survival and inactivation responses of microorganisms at all of the above stages. In addition, there have been considerable developments in the use of finite element analysis for equipment and process-line design, software control of equipment and monitoring, and in the prediction of process reliability. Integration of the above offers the potential to develop new products and processes without the large gross safety margins needed traditionally to ensure safe foods. This approach will be essential if the food industry is to meet the desire of the consumer for less preserved, less heavily processed foods without compromising microbiological safety.

### ASSESSMENT OF RISKS ASSOCIATED WITH FOODBORNE PATHOGENS — AN OVERVIEW OF A CAST REPORT

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Risk assessment methodology was applied to foodborne disease in the United States by a task force convened by the Council for Agricultural Science and Technology (CAST). In modern foodhandling and preparation there is increased chance for contamination and abuse of foods because more handling is involved; there is more time for abuse and possible growth of pathogens, and increased variety of foods may lead to confusion concerning safe and appropriate handling practices. There are new opportunities at the farm, processor, handler and consumer levels which should enhance food safety, including programs being implemented now. The foodborne disease burden (numbers of acute and chronic cases, deaths and costs) is not accurately known and the most recent estimates lag by several years. Thus, estimates of the economic impact of foodborne illness to society and the cost-benefit analysis of control options have a high degree of uncertainty. Still, U.S. foodborne disease is probably costing billions of dollars annually in medical costs and productivity losses. Better identification of pathogens and quantification of risks are essential to identifying the most cost effective methods for improving food safety.

### RISK ANALYSIS AND ITS APPLICATION IN FOOD REGULATION

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Risk analysis has become a well established field in the United States during the past two decades. Risk analysis is used very effectively in several application areas to assess and manage risk. Advances in the application of

risk analysis to a wider range of hazards, including biologic hazards, and scenarios are now occurring. Definitions of risk analysis terms (including risk assessment, risk management and risk communication) have been developed along with the rationale, purpose and need for risk analysis in regulation. Generic principles and applications will be reviewed.

A risk analysis program for foodborne hazards is being developed by the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture. A food risk analysis program for meat and poultry is being designed and some specific projects for risk assessment have been selected. Several types of foodborne hazards (biologic, chemical and physical) will be addressed in the new program. A general overview of risk assessment procedures and the organizational structure for the program will be presented. Integrated national and international food risk analysis activities by the FSIS will be summarized. The Agency expects the new risk analysis program to facilitate the regulation of FSIS inspected food products.

## TECHNICAL SESSION — DAIRY

### VITAMIN FORTIFICATION OF MILK

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The dairy industry has been fortifying milk products with vitamins since the 1940's. Recently, vitamin fortification of dairy products has been questioned by individuals who have conducted analyses of finished products and determined that both over-and-under fortification occur at unacceptable levels. An article in the *New England Journal of Medicine* reported that 80% of the analyzed milk samples contained either 20% less or 20% more than the level of vitamin D declared on the label. The dairy industry continues to scrutinize and seek improvements regarding the addition of vitamins to milk. At the National Conference on Interstate Milk Shipments in 1993, three issues were passed that related to vitamin addition. Dairy companies that fortify with vitamins are now required to keep volume control records. In addition, a new Appendix, which addresses the proper addition of vitamins to milk, was added to the Pasteurized Milk Ordinance. Beginning in 1995, vitamin analysis will be required to be conducted in laboratories certified by the Food and Drug Administration as well. This paper addresses the addition of vitamins to milk, discusses recent data which details testing of milk for vitamin levels, and makes recommendations on how the dairy industry may better control vitamin fortification of dairy products.

### SHELF-LIFE OF COMMERCIAL CONVENTIONALLY PACKAGED COTTAGE CHEESE

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Conventionally packaged cottage cheese collected from six New York State manufacturing plants was subject to microbiological shelf-life analyses. A total of 111 samples, including nonfat, lowfat and creamed varieties were tested for SPC, psychrotroph count, yeast and mold count, gram-negative bacteria, coliform bacteria and sensory evaluation. Samples were tested initially and after 7, 14, 21 and 28 days storage at 6.1 °C. Yeasts/molds and gram-negative bacteria were detected initially in 8% and 18% of the samples, while at day 28 these organisms were detected in 52% and 37% of the samples, respectively. Gram-negative bacteria exceeded 10<sup>6</sup> per gram in 14% of the samples after 28 days. High SPCs were attributed to gram-positive cocci in a number of samples. The level of contamination and spoilage varied among plants though no plant was without failure.

### COMPUTER MODELS FOR THERMAL INACTIVATION OF NATIVE MILK ENZYMES

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In order to support regulations directed towards ensuring the safety of heat processed dairy products, data on inactivation of lactoperoxidase and gamma-glutamyl transpeptidase in whole milk in a pilot plant high-temperature short-time pasteurizer were obtained. A computer program was designed to calculate the integrated lethal effect, or pasteurization effect (PE), at

temperatures of 60-80°C and withholding tubes of 3-60 s. Data were fit to the biphasic logistic equation of Whiting, and models were derived which related values of PE to log percent residual activity. These results suggest that predictive equations based on PE could be used to assess the effectiveness of commercial pasteurization process.

## TECHNICAL SESSION — RISK ASSESSMENT

### APPLICATION OF SEWAGE SLUDGE TO FOOD CROPS

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The Environmental Protection Agency (EPA) enacted new rules for the land application of sewage sludge during 1993. As local waste water treatment plants implement new EPA rules health officials and food industry representatives will need to be able to address questions concerning the use of sewage sludge on food crops. Of special concern to the food industry will be the ability of treatment facilities to reduce pathogens to meet EPA requirements.

Field studies conducted at local waste water treatment facilities demonstrated that EPA pathogen reduction requirements could be achieved at normal operating temperatures and holding times. Window composting temperatures maintained at 55° C for 15 days reduced pathogens below EPA requirements for land application on food crops. The addition of calcium oxide (quicklime) to sewage sludge to generate heat (70 C) and elevate the pH to greater than 12 for more than 72 h also reduce pathogen levels below EPA requirements.

### EFFECT OF HYDROSTATIC PRESSURE, IN COMBINATION WITH HEAT AND/OR IRRADIATION ON THE SURVIVAL OF *CLOSTRIDIUM SPOROGENES* IN CHICKEN

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Growing concerns over the safety of our food supply have prompted the investigation of two processing techniques, high hydrostatic pressure and irradiation, for their effect on microorganisms. Our objectives were to determine the optimum pressure conditions, and also the effect of combining this pressure with heat and/or irradiation, on survival of *C. sporogenes* spores in chicken.

Fresh chicken breast meat was inoculated with 0.1 ml of a 10<sup>7</sup> spore/ml spore suspension in water, and then vacuum-packaged. The samples were shipped to ABB Autoclave Systems, Inc. (Columbus, OH), where they were processed at pressures ranging from 60 to 120,000 psi for 5 to 60 min. Surviving cells were enumerated by plating onto Brain Heart Infusion agar with the number of spores being determined by heating at 80°C for 10 min. Treatment at 100,000 psi resulted in the lowest number of survivors, regardless of the time, compared with the other pressures tested. There was a 7 log<sub>10</sub> reduction in total counts in samples not inoculated with spores, with all samples containing spores showing a baseline of 1 to 3 log survivors, regardless of the treatment.

We then exposed chicken samples to heating at 80°C for 1, 10 and 20 min during pressurization at 100,000 psi. Some samples were exposed to irradiation at 3.0 kGy either before or after pressurization to determine the combined effect on the survival of *C. sporogenes* spores. Irradiation alone had no effect on survival of spores and pressurization at 100,000 psi at 80°C showed maximum reduction after 20 min. Pressurization before irradiation resulted in a lower number of survivors than irradiation before pressurization. This suggests that pressurization in combination with heat and/or irradiation could be used to reduce the number of bacterial spores more effectively than irradiation alone.

### SAFETY AND FOOD EXCELLENCE (S.A.F.E.): A PROGRAM FOR FOODSERVICE WORKERS AND CAREGIVERS, WHO PREPARE FOOD FOR THE CHRONICALLY ILL

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Using a train-the-trainer approach, professionals and paraprofessionals were taught to deliver an education program on safe food preparation to foodservice providers who serve persons with compromised immune sys-

tems. About 474 individuals were trained at six sites in New York and four sites in Colorado. Pre- and post-training questionnaires assessed the knowledge gained by participants, with scores increasing from 87.0-92.7%, respectively. These trained leaders identified one food safety behavior they planned to adopt or strengthen. At three-months post-training, leaders were surveyed to assess their success in meeting their stated behavioral goals. The majority (82% in Colorado and 69% in New York) reported that they adopted or strengthened their selected behaviors "most of the time". These trained leaders then conducted over 112 training programs with more than 3,278 persons who prepare foods for chronically ill people.

#### ENVIRONMENTAL TESTING FOR *LISTERIA*: THE QUANTITATIVE EDGE

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The HACCP emphasis on environmental testing for pathogens requires tests to be extremely rapid, since the microbiological status of processing environments changes continually. Because it omits enrichment, immunomagnetic capture is sufficiently rapid for environmental testing—but is it sensitive? A study submitted to the AOAC Research Institute reported that immunomagnetic capture detected injured *Listeria monocytogenes* 1/2a, 1/2b and 4b more efficiently than a standard cultural method, in environmental samples from inoculated stainless steel surfaces. Ninety-three percent of the *Listeria* in the samples were injured, as shown by inability to grow on medium containing a high concentration of salt. With identical samples analyzed by each method, 94% were positive by immunomagnetic capture and 22% were positive by the cultural method. In a broader study of recovery of *L. monocytogenes* spiked onto seven different environmental surfaces, immunomagnetic capture was at least as sensitive as the cultural method for all sample types. Because immunomagnetic capture omitted enrichment, the number of *Listeria* colonies obtained was related to the original level of contamination. Immunomagnetic capture produced a quantitative result in 24 h, while the cultural method required 3 to 6 days to produce a qualitative result. Immunomagnetic capture is thus more rapid yet as sensitive as a standard cultural method for detection of *L. monocytogenes* in environmental samples. The quantitation it provides can be useful in pinpointing environmental sources of organism.

#### THE PRACTICAL AND EDUCATIONAL ROLE OF ENVIRONMENTAL MONITORING OF FOOD PREMISES

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Monitoring cleaning procedures and preventing occurrence of foodborne diseases is an everyday challenge to our foodservice industry. Our company monitored four food premises in Guelph, Ontario once a week for four weeks. Each week we used five "Culturette" swabs per visit to monitor different critical areas. Each swab was transferred onto a TSA, MacConkey and Rambach agar plate. Our goal was to evaluate the total plate count and the presence, if any, of *Enterobacteriaceae*. Any colonies isolated from MacConkey and Rambach were processed by using the "Microbact System 24E" for identification of *Enterobacteriaceae*.

Results showed both a trend in the quantitative and qualitative evaluation of the test service, and that in Canada this type of service, and most importantly, the educational value in preventing the future cross-contamination in the food preparation facility is a necessity.

#### FOOD FACILITY PLAN REVIEW

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Food facility plan review is a basic requirement of many federal, state and local sanitation codes. The Food & Drug Administration (FDA) 1976 model Foodservice Sanitation Ordinance and 1982 model Retail Food Sanitation Code both contain wording requiring the submission of plans and specifications before foodservice establishment shall be constructed, extensively remodeled, or converted. This standard has been carried forward and is reflected in the new FDA Food Code. A well-planned food production and delivery system can eliminate chaos, waste and inefficiency, and ensure that sufficient and appropriate equipment is available for cooking, holding and serving food. Plans and specifications should be menu driven to reflect the

types and volume of food including preparation; layout; construction materials; finish schedules; equipment types, numbers, location and performance capacities. The FDA Northeast Region Plan Review Development Committee (NRPRDC) developed a guidance document for state & local health authorities. The document was presented for consideration at the 1994 Conference for Food Protection.

#### REGULATORY INSPECTION HACCP VS. FOOD OPERATION HACCP SELF-CONTROL

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Historically, HACCP publications have been written from an inspection and audit perspective by company directors of quality assurance or regulators. Unfortunately, neither the QA director nor the regulator through inspection actually controls the employee performing the food process-related tasks on the line. The president of the company, through and along with the operating staff and supervisors, is the one who makes a zero safety defect process happen. The only effective "inspector" is the employee doing the task. Management accomplishes zero-defect process and employee performance through employee training to mastery of hazard controls in the process and then, leadership and empowerment so that the employees can strive for zero defects. This presentation will discuss the differences between inspection HACCP, often a punishment approach to control, and worker self-control HACCP, a positive consequence approach. It will provide a description of what must be changed and added to inspection HACCP to transform it into a worker hazard self-control prevention improvement program, whereby it is possible for a company to strive for zero liability costs. Examples of successful programs will be discussed.

#### GROWTH OF *SHIGELLA FLEXNERI* IN FOODS: COMPARISON OF OBSERVED AND CALCULATED GROWTH KINETICS PARAMETERS

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*Shigella* causes foodborne gastrointestinal illness; however, little information is available on its ability to grow in foods. Commercially available sterile foods (milk, beef broth, vegetable broth, meats, seafood, vegetables) were inoculated with *Shigella flexneri* 5348 and incubated at 12, 15, 19, 28 or 37°C. Growth curves were fitted from plate count data by the Gompertz equation and generation times and lag times were derived. The observed kinetics values were compared with values calculated using growth models in terms of temperature, initial pH, NaCl and NaNO<sub>2</sub> levels. Observed and calculated values compared favorably for growth at 19 to 37°C. *S. flexneri* grew well in milk at 15 to 37°C but growth at 12°C was variable. The bacteria readily grew in squash (pH 5.3) at 28°C but died off in carrots (pH 5.2). Factors other than those used in growth model may influence bacterial growth in specific foods.

#### TECHNICAL SESSION — ANALYTICAL METHODS

##### COMPARISON OF ENRICHMENT PROTOCOLS FOR USE WITH VIDAS TO DETECT SALMONELLAE

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Different enrichment media were tested in conjunction with the VIDAS automated immunoanalysis system to detect salmonellae. Processed broiler carcasses were rinse sampled and pre-enriched in either buffered peptone (BP) or lactose broth (LB) for 24 h followed by selective enrichment in either selenite cystine (SC), TT or Rappaport for 6 or 24 h followed by post-enrichment in M broth for 6 or 18 h. Overall VIDAS results correlated well with conventional results, but were dependent on the enrichment protocol used. With BP pre-enrichment false negative rates ranged from 14% from the 6 h SC/TT, 18 h M broth to 3% from the 24 h TT, 6 h M broth. With LB pre-enrichment, false negative results ranged from 13% from the 24 h TT, no M broth to 2% from the 24 h TT, 6 h M broth. The VIDAS salmonellae assay is 45 min automated ELISA procedure, which when used with proper cultural enrichment is an effective alternative to conventional procedures for salmonellae detection.



## FLUOROMETRIC ACID PHOSPHATASE METHOD FOR VERIFYING END-POINT TEMPERATURE IN COOKED POULTRY

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Pre-cooked uncured and cured poultry are required to be heat processed to 160°F/71.1°C or 155°F/68.3°C, respectively by the U.S. Department of Agriculture, Food Safety and Inspection Service. A rapid 3 min quantitative fluorescence assay for acid phosphatase (ACP, EC 3.1, 3.2) has been developed for use with water extracts of heated poultry. The method is based on a fluorometric substrate, Fluorophos®, for determining alkaline phosphatase in milk (*J. Food Prot.* 53:588, 1990). Working substrate for the ACP test contains 5mG Fluorophos® in 50 ml acetate buffer pH 5.0 with 10% dimethylformamide. A 75 µL sample of the aqueous meat extract (1 meat: 2 H<sub>2</sub>O) is added to 2.0 ml of substrate in a fluorometer cuvette. The kinetic increase in fluorescence is monitored in a dedicated fluorometer at 38°C and printed after 3 min. Poultry broiler and turkey breast and thigh, marinated broiler breast and thigh, and cured turkey rolls were heated to known end-point temperatures (66.9 to 73.9°C). A quadratic curvilinear decrease in mean ACP activity occurred within each product. Acid phosphatase mean values were different among species within muscle types, marination, and curing. This procedure provides a rapid, sensitive analytical method for verifying HACCP, FSIS or FDA regulatory end-point temperatures as lethal interventions for pathogen reduction in poultry products.

## IMPROVED MEDIUM AND METHOD FOR GROWING *ESCHERICHIA COLI*

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With the recent outbreaks of *Escherichia coli* O157:H7 foodborne intoxication, there is increased need for an economical overnight assay to show presence/absence of these organisms in food. The purpose of this study was to maximize the growth of *E. coli*, while inhibiting the growth of competing flora. Using mEC broth as a basal medium, the effect of temperature (37, 40, 42°C), novobiocin concentration (0, 7.5, 20 mg/L), agitation (100 RPM), media additives and dye indicator systems were evaluated in pure cultures and food samples. In 18 to 24 h, the modified medium allowed for growth of *E. coli* equal to that of TSB without inhibitors, while suppressing the growth of all but a few closely-related organisms. Optimal growth of *E. coli* with maximum inhibition of competing flora (10<sup>5</sup> CFU/ml) was achieved at 37°C shaking or 42°C non-shaking (<1 CFU/ml *E. coli* to 10<sup>9</sup> CFU/ml in 18 to 24 h), with 20 mg/L novobiocin. 37°C non-shaking and 42°C shaking conditions were not acceptable because of variable color change and overgrowth of competing flora (*Proteus/Enterobacter* 10<sup>9</sup> CFU/ml in 18-24 h). The new medium had the additional advantage of a color change by means of a dye indicator system, which signaled a potential *E. coli*-containing sample thereby eliminating the testing of non-*E. coli* containing samples.

## COMPARISON OF A MICRO IDENTIFICATION SYSTEM TO CONVENTIONAL BIOCHEMICAL PROCEDURES FOR IDENTIFICATION OF *SALMONELLA*, *ESCHERICHIA COLI* AND OTHER GRAM-NEGATIVE ENTEROBACTERIACEAE FROM FOOD ORIGIN

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Several miniaturized systems for the biochemical identification of microorganisms have been developed. These systems have gained wide popularity because of their ease of use and convenience. A new system that incorporates both traditional tests for fermentation, degradation, oxidation and hydrolysis with enzymatic characteristics is currently available. The objective of this study was to determine if the new system could correctly identify members of the family *Enterobacteriaceae*.

One-hundred-and-ninety-five *Salmonella* sp. of food origin were identified using BAM/AOAC biochemicals and the miniaturized system. Each isolate was identified approximately five times for a total of 979 isolates. The correlation between the two procedures was 100%. Non *Salmonella Enterobacteriaceae* were also tested with seventy-nine strains representing *Vibrio* sp., *Enterobacter* sp., *Proteus* sp., *Citrobacter* sp., *Hafnia* sp., *Pseudomonas*

sp., *Escherichia* sp., *Providencia* sp., *Yersinia* sp., *Serratia* sp., *Edwardsiella* sp. and *Shigella* sp.

A total of 392 isolates were analyzed with the BAM/AOAC method and the miniaturized system. A 99% agreement between the two methods was observed. This data indicates that the miniaturized system is equivalent to the traditional BAM/AOAC biochemical system for the identification of gram-negative *Enterobacteriaceae* in foods.

## A NEW RAPID COLIFORM DETECTION METHOD, PETRIFILM 2000 COLIFORM COUNT PLATE

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A new rapid method for the detection and enumeration of coliforms has been developed. The Petrifilm 2000 Coliform Count Plate (P2000CC) has been formulated to detect presumptive coliforms in food and dairy products as early as 6 h after incubation. Confirmed coliforms, indicated by acid and gas production from lactose, were detected as early as 8 h. Food samples with potential catastrophic contamination could be detected in 4 h.

This paper describes our work on the performance of the P2000CC plate with a variety of food and dairy products. A total of 67 samples was tested. A greater than 90% correlation was found with the rapid result as compared to the 24-h violet red bile agar result.

## A MURINE MONOCLONAL ANTIBODY SPECIFIC TO O-SEROGROUP *SALMONELLA*

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In the past 10 years, incidence of *Salmonella enteritidis* as a cause of human salmonellosis has increased dramatically, worldwide. Although this pathogen is the most frequently isolated D-serotype from poultry, eggs and egg products, other D-serotypes such as *Salmonella berta* were recently reported to be responsible for outbreaks of poultry-borne salmonellosis in Europe and Australia. The objective of this study was to develop a specific monoclonal antibody (MAb) that could be used as a probe to detect serogroup D salmonellae in foods. A murine MAb (IgG<sub>2a</sub>) was produced by a fusion of P3X63-Ag8.653 myeloma cells with splenocytes of mouse immunized with attenuated *S. enteritidis* cells. The specificity of the MAb was determined for 29 *Enterobacteriaceae*, including 24 salmonellae representing ten serogroups, using a noncompetitive ELISA. The MAb was specific to D-serotypes salmonellae exhibiting highest reactivity with five phage types of *S. enteritidis* (1,4,8,13 and 13a). In addition, the MAb proved to be reactive with a distinct epitope (factor 9) present on the antigen O of lipopolysaccharide isolated from *S. enteritidis*.

## ATP LUMINESCENCE AS A MEANS TO RAPIDLY DETECT MICROBIAL AND FECAL CONTAMINATION ON CARCASS TISSUE

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ATP Luminescence (Bioluminescence) was evaluated as a means to rapidly detect gross microbial contamination from feces on bovine carcass surface tissue (BCT). Microbial ATP was selectively distinguished from non-microbial ATP by the assay procedure which took 1 h to complete. Regression analysis of microbial ATP and viable count scattergrams showed lean and adipose BCT artificially contaminated with bovine feces had the same regression line parameters ( $p > 0.05$ ) and therefore the microbial ATP response was similar from either tissue type. Correlation coefficients (R) of these regression lines were  $> 0.90$ . Microbial ATP assay method results from carcass swab samples responded linearly from approximately  $\log_{10}^3$  to  $\log_{10}^8$  CFU/cm<sup>2</sup> of total bacteria (aerobic and anaerobic, 37°C). Results indicated that swab samples can be held at 5°C for up to 6 h without compromising ATP Luminescence results. Samples taken from tissue held at 5°C for up to 6 h showed no statistically different ( $p > 0.05$ ) ATP Luminescence results from 0 to 6 h. A modified assay procedure shortened the assay time from 1 h to 20 min. Both the standard and modified ATP Luminescence methods show potential for use as a means to rapidly determine fecal contamination on red meat carcasses as well as an index for determining effectiveness of carcass reconditioning procedures.

## RAPID ASSESSMENT OF LISTERIA CONTROL USING BIOLUMINESCENCE

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With the developing market in Europe of short shelf-life chilled products, *Listeria* has come to prominence as a major pathogen of concern. Of utmost importance to the food industry, therefore, is the selection of disinfectants which are capable of controlling growth of this organism. *Listeria monocytogenes* 23074 was engineered to express *Lux AB* genes to give a bioluminescent phenotype. Conditions were manipulated in order to maximize light output from the organism which was subsequently correlated with cell viability. Viable count measurements (24 h) and bioluminescence (5 min) were carried out in parallel to assess the suitability of bioluminescence to monitor the effectiveness of a range of disinfectants. Concentration exponents calculated for each biocide from the viable count and bioluminescence data were shown to correlate well. In all cases, bacteria in suspension were more susceptible to biocide challenge than those attached to surfaces. The rapidity of bioluminescence constitutes a major advancement in testing of biocide effectiveness upon bacteria in suspension and on surfaces. In surface tests, the light output can be monitored 'in situ', alleviating the need to remove the organisms. In industry, bioluminescence could be used as part of a HACCP system to monitor and control biocidal potential in a time more closely related to in-process control.

## EFFECT OF MONOLAURIN ON *L. MONOCYTOGENES* SCOTT A AT 37 AND 8° C

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Monolaurin (ML) at 0.0073 mM was tested for ability to kill large populations ( $10^6$  to  $10^8$  CFU/ml) of *Listeria monocytogenes* Scott A in tryptic soy broth (TSB) as a function of growth temperature (GT) and exposure temperature (ET) at 10 or 37°C. Cells were grown at the prescribed temperature in TSB to late log phase, harvested and resuspended in fresh TSB to about  $10^8$  and  $10^6$  CFU/ml for the higher and lower GT used. After 2 h the ML was added and viable counts were determined by sampling and plating cells at h 0, 2 and 3. Exposed cells were tested for injury versus death by using basal and 1-h later overlays of the following three combinations: TSAYE-TSAYE, MOX-TSAYE, MOX-MOX. Exposure temperature was more important than GT since an ET of 37°C killed 4-5 logs of L.m. cells when the GT was 10 or 37°C. Conversely, at an ET of 10°C only 0.5 to 1 log of L.m. cells were killed in 1 h when the GT was 10 or 37°C. Resuscitation experiments indicated exposed cells were killed rather than injured by ML.

## AN ISOLATION METHOD FOR *ARCObACTER BUTZLERI* FROM POULTRY

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*Arcobacter (Campylobacter) butzleri* belongs to a newly-designated genus of bacteria that were previously regarded as "aerotolerant" campylobacters. *Arcobacter* spp. have been isolated from aborted and diarrheic animals, and recently *A. butzleri* has been associated with human enteritis. The organism is found in water and sewage, and has been isolated from animals, birds, meats and poultry. To assess the prevalence of *A. butzleri* in fresh poultry, a procedure used to isolate thermophilic campylobacters was modified to selectively recover *A. butzleri*. The method included enrichment in Rosef's Broth, modified by omitting the antibiotics routinely used to select for campylobacters, and relying on cefepazone (32 mg/L) for inhibition of competitive microflora. Poultry carcass rinses were incubated in modified Rosef's Broth at 30°C under microaerobic conditions for 48 h, filtered through low-protein-binding syringe filters (0.45 µm) and the filtrates spotted-cultured on charcoal agar (modified CCDA). The plates were incubated aerobically at 30°C for 48 to 96 h. Pure cultures of *A. butzleri* were obtained, confirmed by Lior's *Arcobacter* biotyping and serotyping schemes. Poultry appears to be a significant reservoir of the organism: *A. butzleri* was isolated from all of the 40 carcasses tested to date. This procedure also allowed the isolation of *A. butzleri* from ground chicken and turkey meat. Further epidemiologic studies will lead to a better understanding of the role of foods in the transmission of *A. butzleri*.

## IMPROVED ENRICHMENT RECOVERY OF *CAMPYLOBACTER* SPP. FROM BROILER CHICKEN CARCASSES

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Recovery of *Campylobacter* spp. from retail broiler chicken carcasses by an enrichment method described in both the *Food and Drug Administration Bacteriological Analytical Manual* and in the *Food Safety Inspection Service Microbiological Laboratory Guidebook* was compared with an enrichment method described by Bolton. One-hundred-and-twenty carcasses were rinsed in buffer and the associated bacteria were pelleted and resuspended. One-milliliter undiluted and 1-ml of a 1:1,000 dilution of the suspension were transferred into both enrichment broths and cultured according to specifications. The official method recovered the organism from 29 of 120 of the undiluted samples and 1 of 120 from the diluted samples, while the Bolton enrichment method recovered *Campylobacter* spp. from 65 and 15 of these same carcass samples. The methods differed significantly from one another in their recovery of *Campylobacter* spp. This work points toward a new and improved method for the recovery of *Campylobacter*.

## DNA PROBES-HGMF METHODS TO DETECT ENTEROHEMORRHAGIC *E. COLI* AND *SHIGELLA* IN FOODS

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PCR products were labeled with digoxigenin and these probes hybridized with the exposed DNA on hydrophobic grid membranes (HGMFs). Such probes were prepared for both enterohemorrhagic *E. coli* (EHEC) and *Shigella*. Enterohemorrhagic *Escherichia coli* produce verotoxins and adhesins that are important agents in pathogenesis of the disease. *Shigellae* require an invasive gene which is located on a plasmid. The verotoxin gene DNA probe-HGMF method was used to detect 135 of 142 (95%) VTEC strains inoculated into ground beef. In addition, strains added to vegetables and apple cider were recovered, but the cider interfered with both the DNA probe and PCR methods, unless it was diluted at least 1:100. No VTEC were found in 45 vegetables and cider samples from across Canada. One of 27 wild animal intestinal contents, however, contained a VTEC. Preliminary studies on two probes derived from PCR products for the adhesin gene show promise in differentiating VTEC from non-VTEC organisms. A DNA probe for the invasive gene in *Shigella* spp. detected 25 *Shigella* strains and 5 enteroinvasive *E. coli* (EIEC) strains in pure culture. Over 95% of these strains were detected by the probe in artificially-inoculated 2% milk, turkey luncheon meat and chopped green cabbage.

## TECHNICAL SESSION — ANTIMICROBIALS

### DECONTAMINATION OF BEEF CARCASS TISSUE WITH BACTERIOCINS USING A MODEL CARCASS WASHER

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Bacteriocins can effectively inhibit or suppress the growth of undesirable bacteria in a variety of foods, including red meat products. Given the potential use of bacteriocins as biopreservatives in various food products, the following study was undertaken to determine the effectiveness of spray washing the surface of beef carcass tissue with bacteriocins to inhibit gram-positive bacteria. Sections of lean and adipose tissues from the surfaces of beef carcasses were inoculated with approximately  $4 \log_{10}$  CFU/cm<sup>2</sup> of *Brochothrix thermosphacta*, *Carnobacterium divergens* or *Listeria innocua*. Following spray treatments with water or bacteriocin, bacterial populations were enumerated and again after incubation for 24 h at 4°C. Spray treatments with water did not significantly alter the bacterial populations at either day (<log<sub>10</sub> reduction). However, spray treatments with nisin (5000 AU/ml), significantly affected log reductions of 1.79 to 3.54 log<sub>10</sub> CFU/cm<sup>2</sup> at day 0, and from 1.97 to 3.60 at day 1. The data indicate that spray washing is an effective means of applying bacteriocins and that these antimicrobial compounds may be useful as sanitizers of red meat carcasses. Research is in progress to determine if the biological effects of bacteriocin spray treatments

can be combined with the preservation effects of vacuum-packaging to enhance the microbiological safety and extend the shelf-life of red meat.

#### EVALUATION OF METHODS TO DELIVER BACTERIOCINS DURING WIENER MANUFACTURING FOR CONTROLLING *LISTERIA MONOCYTOGENES*

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The antilisterial activity of bacteriocins (enterocin, sakacin, pediocin or a "cocktail") was evaluated at different stages during manufacture of beef wieners. Bacteriocins (0-20,000 arbitrary units [AU]) were blended into wiener batter, surface-applied to links by dipping, or sprayed in agar or gelatine onto packaging film. Links were surface-inoculated with a 4-strain mixture of *Listeria monocytogenes*; (ca.  $10^4$  CFU/pkg), vacuum-packaged, and stored at 25°C or 4°C. In control samples and in samples containing  $\leq 10,000$  AU/ml in agar film, counts of *L. monocytogenes* increased about  $3 \log_{10}$  CFU/pkg within 2 days (25°C) or 4 weeks (4°C). In contrast, *L. monocytogenes* counts fell below detection (50 CFU/pkg) in the following treatments; i) within 1 day (25°C) or 2 weeks (4°C) using  $\geq 10,000$  AU/g batter; ii) within 1 d (25°C) using  $\geq 10,000$  AU/ml or 2 weeks (4°C) using 5,000 AU/ml by dipping; and iii) within 1 day (25°C) using  $\geq 5,000$  AU/ml or 4 weeks (4°C) using 20,000 AU/ml gelatin film. These data establish that application strategies can influence the levels of bacteriocins required to control *L. monocytogenes* in vacuum-packaged wieners.

#### CHEMICAL AND MICROBIOLOGICAL QUALITIES OF RESTRUCTURED VACUUM-COOKED LAMB ROASTS CONTAINING SODIUM OR POTASSIUM LACTATES

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The effects of sodium lactate (SL) or potassium lactate (PL) on the chemical and microbiological qualities of restructured vacuum-packaged lamb roasts were studied. Restructured lamb roasts with 0%, 1% or 3% SL or PL (pH 6.2) were prepared using fresh lamb meat. Lamb roasts were cooked to an internal temperature of 70°C. Cooked roasts were stored overnight at 2 to 3°C and cut into slices (0.5 cm thick). Each slice was vacuum-packaged individually and stored at 2 to 3°C for 0, 20, 40, 60 and 80 days. Samples were analyzed for pH, water activity ( $a_w$ ), thiobarbituric acid (TBA), and aerobic and lactic acid bacteria counts on each storage day. In a similar study, *Salmonella typhimurium*, *Escherichia coli* O157:H7 and *Listeria monocytogenes* were inoculated individually onto the surface of roast slices, and vacuum-packaged and stored at 4°C. Growth behavior of the pathogens was evaluated on each storage day mentioned above. Addition of SL or PL reduced the pH,  $a_w$  and TBA values of the lamb roasts ( $P < 0.01$ ). Total aerobic bacteria counts were lower ( $P < 0.01$ ) in samples containing 1% or 3% SL or PL, however, lactic acid bacteria numbers were reduced ( $p < 0.01$ ) only by 3% SL or PL. Treatments had no effect ( $p > 0.05$ ) on color  $L^*$ ,  $A^*$  and  $b^*$  values. Numbers of *L. monocytogenes* were reduced ( $p < 0.01$ ) by 3% SL or PL. However, compared with untreated controls, the numbers of *S. typhimurium* and *E. coli* O157:H7 were less affected by treatments ( $P < 0.05$ ) and were reduced mainly as a result of storage time.

#### GROWTH INHIBITION OF *PENICILLIUM* SPECIES BY LACTIC ACID BACTERIA

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Lactic acid bacteria (LAB) are universally known to be present in many raw food products, and they are involved in the manufacturing and preservation of many fermented products. In this study, several LAB strains were isolated from various food products using selective plating procedures. To test for the antimycotic activity, a specific volume of the LAB supernatant was placed on sterilized discs and placed on PDA plates seeded with a known number of mold spores. The diameter of the inhibition zones was measured after incubation of the PDA plates. The mold strains tested were, *Penicillium italicum*, *Penicillium cyclopium*, *Penicillium citrinum*, *Penicillium expansum*, *Aspergillus flavus* subsp. *parasiticus* and *Aspergillus ochraceus*. Several LAB isolates had no inhibitory effect on any of the mold strains. Some of the LAB isolates inhibited the growth of *Penicillium* species without affecting

the growth of *Aspergillus* species. The antimycotic activity of two LAB isolates was shown to be unrelated to the production of lactic acid or of hydrogen peroxide. This suggests the possibility of the presence of antimycotic substances in the supernatants. Thus, the potential of using LAB or their antimycotic by-products to control *Penicillium* growth in food products may be promising.

#### MECHANISM OF INHIBITION OF AFLATOXIN BIOSYNTHESIS BY *LACTOBACILLUS CASEI PSEUDOPANTARUM*

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*Lactobacillus casei pseudopantarium* isolated from a silage inoculant was found to inhibit aflatoxins B1 (AFB1) and G1 (AFG1) biosynthesis by *Aspergillus flavus* subsp. *parasiticus* NRRL 2999. The inhibitory activity in the *Lactobacillus* cell free supernatant was found to be sensitive to proteolytic enzymes such as trypsin and  $\alpha$ -chymotrypsin. The location of the inhibitory site in the biosynthetic pathway of aflatoxin was investigated by using the tip culture method, aflatoxin intermediates and a blocked mutant species of *A. flavus*. When sterigmatocystin was added to the system, the levels of AFB1 and AFG1 increased by 78% and 73%, respectively. When O-methylsterigmatocystin was fed into the system the levels of AFB1 increased by 58%. In *Lactobacillus* cell free supernatant, without precursors, there was production of versicolorin A. Addition of NADPH and S-adenosylmethionine to the cell free mycelial extract restored production of AFB1 and AFG1. The results suggest that the inhibition of aflatoxins occurred between the versicolorin A and sterigmatocystin intermediates. The inhibition may be due to an inactivation of the enzymatic system required for the biosynthesis of sterigmatocystin and AFB1.

#### OPTIMIZATION OF PARAMETERS FOR PRODUCTION OF NISIN AND INHIBITION OF *LACTOBACILLUS PLANTARUM* IN A MODEL MIXED-CULTURE FERMENTATION

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Nisin-resistant *Leuconostoc mesenteroides* NCK 293 and nisin-producing *Lactococcus lactis* NCK 401 were previously shown to inhibit *Lactobacillus plantarum* ATCC 14917 in a model sauerkraut fermentation under optimal processing conditions (2% NaCl, 20°C). Because salt concentration and temperature are not easily controlled in a commercial sauerkraut operation, the effect of suboptimal process conditions on the production of nisin and inhibition of *L. plantarum* was determined using a statistically designed experimental protocol. Various temperatures (10 to 30°C) and salt concentrations (0 to 3.4%) were evaluated. Individual strains were enumerated using selective agars and incubation temperatures. Isogenic nisin-negative *L. lactis* NCK 402 was used in control fermentations to ensure that inhibition was due to the production of nisin and not other factors. The time for *L. plantarum* to decrease to below detectable levels was determined and used to generate a model for the mixed starter culture system. The model was verified with fermentation conditions not used in the original protocol. Both salt concentration and temperature had a significant influence on the production of nisin and inhibition of *L. plantarum*. Nisin could be produced at levels adequate to inhibit *L. plantarum* within 24 h at all temperatures tested for salt concentrations between approximately 1.4 and 2%.

#### CONTROL OF *SALMONELLA*, *LISTERIA MONOCYTOGENES*, *CAMPYLOBACTER JEJUNI* AND PSYCHROTROPHS ON SKIN WITH LACTIC ACID AND SODIUM BENZOATE

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The use of lactic acid and sodium benzoate to inactivate *Salmonella*, *Listeria monocytogenes*, *Campylobacter jejuni* and psychrotrophs on chicken skin was studied. Skins inoculated with these pathogens were washed with sterile water, 0.5% lactic acid/0.05% sodium benzoate (LB55), or 0.3% lactic acid/0.05% sodium benzoate (LB35) and stored at 4°C for up to 16 days. Populations of *Salmonella*, *L. monocytogenes* and *C. jejuni* detected on skin after washing with water were  $\log_{10}$  4.0, 4.7, and 3.5 CFU/cm<sup>2</sup>, respectively, while populations on skin washed with LB55 or LB35 were about 1.0  $\log_{10}$

less. Viable *Salmonella* on control skin decreased slightly to  $\log_{10}$  3.5 CFU/cm<sup>2</sup> after 8 days. No viable *Salmonella* were detected on LB55- and LB35-treated skin after 2 and 8 days of storage, respectively. The population of *L. monocytogenes* on control skin increased to  $\log_{10}$  5.3 CFU/cm<sup>2</sup> after 8 days, whereas none was detected on LB55- and LB35-treated skin after 6 days. No *C. jejuni* were detected on LB55- or LB35-treated skin after 2 days. Psychrotrophs increased from  $\log_{10}$  3.2 to  $\log_{10}$  8.4 CFU/cm<sup>2</sup> within 8 days on control skin and to  $\log_{10}$  4.0 CFU/cm<sup>2</sup> on treated skin after 16 days of storage. This study indicates that LB55 or LB35 can be used as wash solutions to kill and/or subsequently prevent the growth of *Salmonella*, *L. monocytogenes*, *C. jejuni* and psychrotrophic bacteria on chicken skin.

#### **INFLUENCE OF SODIUM CHLORIDE ON THERMAL INACTIVATION AND RECOVERY OF NON-PROTEOLYTIC *CLOSTRIDIUM BOTULINUM* TYPE B SPORES**

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Demand for minimally processed (pasteurization temperatures) refrigerated foods with reduced salt has resulted in significant safety concerns against the survival and growth of non-proteolytic *Clostridium botulinum* type B spores. Therefore, the heat resistance of non-proteolytic *C. botulinum* type B spores was determined at 75, 80, 85, and 90°C. Heat-shocked spores were inoculated in turkey slurry containing 0, 1, 2 or 3% salt. Heated spores were enumerated on both Reinforced *Clostridium* Medium (RCM) with lysozyme and on the same conditions as the heating medium. D-values in turkey slurry containing no salt were 31.97 min at 75°C, 15.21 min at 80°C, 4.85 min at 85°C and 0.80 min at 90°C. D-values increased with 1% salt in the heat medium when the recovery medium contained no salt. Apparent or measured heat resistance was decreased with increasing salt concentration in both the heating medium and the recovery medium.

#### **A FIELD STUDY EVALUATING THE EFFECTIVENESS OF DIFFERENT HAND SOAPS AND SANITIZERS**

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Plain hand soaps, antimicrobial hand soaps, E2 rated hand soaps and instant hand sanitizers were evaluated in a foodservice setting to determine their effectiveness in reducing bacteria on hands. The results showed that the three types of hand soaps were effective using a twenty second handwash procedure. The E2 rated hand soaps were significantly (90% confidence) more effective in reducing bacterial numbers than the plain or antimicrobial hand soaps. The instant hand sanitizers resulted in a significant increase in bacterial numbers on hands and may, therefore, be counterproductive for use in the foodservice industry.

#### **DEVELOPMENT OF BACTERIOCIN-BASED PACKAGING TO REDUCE PATHOGENIC ORGANISMS IN FRESH POULTRY**

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This study tested the efficacy of a bacteriocin-coated packaging film delivery system to reduce *Salmonella* contamination of fresh poultry. Three packaged films of varying hydrophobicities were evaluated. Three formulations (pH 3.5-3.8) composed of 100 µg/ml nisin and varying concentrations of citric acid, EDTA and Tween 20 were sprayed onto 2 cm<sup>2</sup> package films and applied either wet or dry to 2 cm<sup>2</sup> samples of broiler drumstick skin inoculated with a nalidixic acid-resistant (NA) strain of *Salmonella typhimurium*. After incubation at 4°C for 24 h, the number of surviving organisms were recovered from the skin and package film using a rinse procedure and enumerated on BHI agar containing 800 ppm NA. Log reductions in *S. typhimurium* population were calculated between treated and untreated samples and ranged from 0.40 to 2.06. In general, wet films resulted in higher kills than films containing air-dried nisin formulations. The level of kill was also influenced by the type of film. Compositional variations of the nisin-containing formulations significantly affected the level of kill. These findings show that the addition of bacteriocin-based formulations to primary

packaging films may be effective in reducing the level of pathogenic and spoilage organisms on fresh meat products.

### **MICROBIOLOGY VS EPIDEMIOLOGY: COMPLEMENTARY OR INCOMPATIBLE DISCIPLINES SYMPOSIUM**

#### **WORLDWIDE SURVEILLANCE OF FOODBORNE DISEASE BASED ON EPIDEMIOLOGICAL AND MICROBIOLOGICAL FINDINGS**

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Microbiologists like to see hard data before drawing any conclusions about experiments, such as counts, pH or  $a_w$  values, whereas epidemiologists often obtain their information from surveys and investigations of illnesses, with trends over time. Can these two disciplines be combined in a synergistic way to reveal new information, or are they mutually exclusive? And can appropriate policies be developed from combined microbiological and epidemiological data? The two fields covered are food and environmental bacteriology.

#### **MICROBIOLOGY VS. EPIDEMIOLOGY: "WHO DO YOU TRUST?"**

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In order to detect an outbreak of foodborne illnesses, several factors must occur. Persons must be sick enough to seek medical care or mad enough to call their local health department. Often enough persons must become ill within a short period of time to catch the doctor's attention and prompt him or her to report the cases. In a few cases, a laboratory may notice an unusual number of isolates or positive serologies from a given area and notify a health department.

State health departments are increasingly becoming more proactive in assuring a safe food supply. Microbiological testing is conducted either on 1) random samples of a specific food (e.g., *Escherichia coli* O157:H7 in beef), 2) the environment of the food source (water from oyster collection sites) or 3) the general population (sewage sampling for cholera). These efforts have been met with mixed success.

The talk will look at the recent experience of the Texas Department of Health in the detection, investigation and control of foodborne outbreaks and the role that the state public health laboratories play in these efforts.

#### **HUMAN AND ARMADILLO LEPROSY IN THE SOUTHERN UNITED STATES**

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When the U.S. autochthonous cases of human leprosy are plotted by county or parish of birth, they cluster along the Texas and Louisiana coastline, and along the southern reaches of the Mississippi and Red Rivers; incident cases elsewhere are singular and sporadic. This distribution reveals a geographical relationship to warm, moist air and soils. Soil moisture can be observed as an inverse function of the difference between the night and day surface temperatures as measured by NOAA VHRR satellites; the wetter the soil the smaller the temperature difference. The prevalence of *Mycobacterium leprae* infections in the nine-banded armadillo, *Dasypus novemcinctus*, is similarly distributed in the southern Gulf States of the U. S.; seropositive armadillos are not found in dry areas but only along the Texas-Louisiana Gulf coast and in the inland moist soils of the 'Delta.' In recent decades new leprosy cases have uniformly failed to have a prior family or contact history of leprosy. While there is a high frequency of handling armadillo carcasses among newly diagnosed human cases, this may be a cultural convergence as armadillos are commonly eaten in southern Louisiana and Texas. As the geographic distribution of moist soils is similar to the distribution of high relative humidity, we may be observing from space the survival of *M. leprae* in humid air as a risk to humans and its survival in moist soil as the risk to armadillos. The present state of development of this project is to better determine the statistical relationship of the thermal inertia of remotely sensed ground (using AVHRR) in relation to soil moisture deficits (using irrigation models), soil type/geology, and vegetation cover (or vegetation index). Hypothesis verification will be done in Venezuela because of the high quality of its national leprosy register and the presence of *Dasypus novemcinctus* in

the country. Human leprosy in Venezuela is clustered along the Orinoco River, and especially in its western and southernmost reaches.

#### **A MICROBIOLOGICAL PARADOX: VIABLE BUT NON-CULTURABLE BACTERIA**

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Results from work in our laboratory over the past 15 years have shown that many gram-negative microorganisms enter into a viable but nonculturable state when exposed to adverse environmental conditions for growth and replication. The parameters most closely associated with induction of the viable but nonculturable phenomenon include, for example, salinity, temperature, pressure and nutrient concentration. Viability of cells present in environmental samples has been demonstrated using a variety of techniques, including treatment with nalidixic acid and subsequent staining with acridine orange, uptake of radio-labelled substrates, and induction of enzymes involved in a variety of metabolic pathways. The potential pathogenicity of these organisms, specifically *Vibrio cholerae*, *Escherichia coli*, *Salmonella* spp., and *Shigella* spp. has been demonstrated employing animal models. Human volunteer data, using a vaccine strain of *Vibrio cholerae* have shown the ability of viable but nonculturable bacteria to cause diarrhea. Based on these observations and the increasingly large body of knowledge on survival of bacteria, particularly under adverse environmental conditions, including starvation, stationary phase growth, dormancy and other survival strategies, leads to the conclusion that culturability as an index of viability can no longer be relied upon. From the public health perspective, it is important to develop testing methods for effective monitoring of bacteria in food and water. Monoclonal antibody and gene probe methods are proving useful. Clearly, a new paradigm must be constructed, regarding viability, survival and death of bacteria.

#### **HAZARD ANALYSIS: THE LINK BETWEEN EPIDEMIOLOGY AND MICROBIOLOGY**

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Case control studies provide data upon which to form hypotheses about vehicles responsible for the foodborne illness. Laboratory analyses of specimens from the ill confirm etiologic agents and of samples of implicated foods prove the hypotheses. But, yet for a foodborne disease to occur, the food must become contaminated and if microorganisms are responsible, the contaminant often survives processing or preparation and often it proliferates during storage. Hazard analysis can detect the source and mode of contamination, whether pathogens survived or would have been inactivated during processing, and whether bacteria multiplied to an extent that sufficient populations proliferated or toxins generated to cause illness. This can be done despite inadequate epidemiological data or when suspect foods are not available for collection, the wrong foods or portions less contaminated are collected, or where inappropriate testing is done. This paper describes the role of hazard analysis in identifying contributing factors during outbreak investigations and in predicting risks that food operations will lead to such consequences.

#### **MICROBIOLOGY, CHEMISTRY AND EPIDEMIOLOGY: THE SETTING OF FOOD SAFETY POLICY**

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The establishment of public policy is a complex of many factors. This is particularly true for health related areas such as food safety, where science is assumed to play a fundamental role in the establishment of such policy. In reality the uncertainties of contemporary food safety science have resulted in its use as a screen behind which decisions are made based primarily on non-scientific factors. Chemicals in the food supply, for example, receive much higher priority than do microbiological hazards in the implementation of national health policy. There are several reasons for this, not the least of which, until recently, has been difficult in the identification of foodborne pathogens. Equally important, credible epidemiological data concerning foodborne disease has been difficult to develop, particularly in those countries, such as the United States, where mandatory reporting of most outbreaks

is not required. For chemical hazards, epidemiologic data has not proven useful because of the inherent insensitivity of such data which tends to reduce its value in the debate over food safety. It is clear, therefore, that the development of a national public health policy must depend not only on improvement of the science, but also on the improvement of the communications of that science to the public and those who are responsible for the establishment of public policy. The underlying attitudes of scientists in public health fields also plays an important role. There is no dichotomy between "microbiology and epidemiology." (In fact, in the future development of epidemiology, the increase in the sensitivity and markers that provide more sensitive and more meaningful responses to the impact of environmental variables.) The single most important activity of public health scientists must be the development of a consensus that reduces uncertainty and, therefore, reduces the number of options that policy makers have in establishing national and international health policies.

#### **TECHNICAL SESSION - GENERAL FOOD MICROBIOLOGY**

##### **INCIDENCE OF *ARCOCACTER* SPP. IN GROUND PORK**

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Aerotolerant *Campylobacters* were first isolated in the late 1970's by Ellis et al. from aborted bovine fetuses. These organisms can grow in the presence of atmospheric levels of oxygen, are spiral or vibrio-like in shape, and are able to grow at 15°C. Recently, rRNA studies have redefined the categorization of these species of *Campylobacter*, resulting in their being classified into a new genera, *Arcobacter*.

These aerotolerant strains have been implicated in human diarrheal cases involving roast beef in France, and most recently in an outbreak of recurrent abdominal cramps in Italy.

The objective of this study was to determine the prevalence of *Arcobacter* spp. in ground pork, in an effort to ascertain the likelihood of similar outbreaks occurring in the United States.

Samples of ground pork were taken from an Iowa pork slaughtering facility over the course of one week in June, 1993. Ten grams of each sample were introduced to 50 ml plastic centrifuge tubes containing 20 ml P-80 semisolid *Arcobacter* selective medium and incubated at 30°C for 9 days. A portion of each sample was placed on BHI agar supplemented with 10% defibrinated blood, CVA agar and modified *Yersinia* selective agar (CIN<sup>+</sup>). Each plate was streaked for isolation and incubated under microaerophilic conditions at 30°C for 48 h. Isolated presumptive positive colonies were grown on BHI agar and genetically probed for identification using the cell suspension dot blot procedure.

Of the 139 samples of ground pork that were assayed, 89.2% were positive for *Arcobacter* spp. The CVA agar showed a 91.4% isolation efficiency, that is, the percent of positive isolates on a particular media that come from known positive *Arcobacter* samples. The CIN<sup>+</sup> medium showed efficiency at 83.5% while the BHI with 10% blood only had an isolation efficiency of 54.0%. CVA provided only seven samples that were positive only on that medium while CIN<sup>+</sup> showed three samples where it was the sole isolating medium. Also, 78.4% of the positive samples were positive both on the CVA and CIN<sup>+</sup> medias.

The high prevalence of *Arcobacter* spp. in ground pork points to a concern over the potential for outbreaks by this organism in this product.

##### **COMMERCIAL FIELD TRIALS DEMONSTRATING SALMONELLAE REDUCTION IN BROILERS USING A MUCOSAL COMPETITIVE EXCLUSION TREATMENT**

Nelson A. Cox,\* Microbiologist, J. S. Bailey and N. J. Stern, U.S. Department of Agriculture, ARS, Russell Research Center, P. O. Box 5677, Athens, GA 30613

A two-step treatment of broiler chicks with a mucosal competitive exclusion culture (MCE) was tested. The MCE was first sprayed on chicks in the hatchery and also provided in the first drinking water. Commercial flocks were treated and compared with parallel, untreated control flocks in Puerto Rico and Georgia. After grow-out, carcass rinse samples of fully processed birds were analyzed. In Puerto Rico, salmonellae prevalence in processed carcass rinses were significantly reduced (P<0.05) from 41% in control flocks to 10% in treated flocks. In Georgia very few salmonellae were

found in Trials 1 and 2 which made it difficult to assess efficacy. In Trial 3, 16% of the processed carcasses from the control group were salmonellae positive as compared to only 2.7% in the treated group. These field trials demonstrate that MCE can serve as a useful and effective means to reduce salmonellae contamination of commercial processed broilers.

#### THE ATTACHMENT OF VIABLE AND NON-VIABLE *SALMONELLA* TYPHIMURIUM TO POULTRY SKIN

K. Y. Kim,\* Research Associate, H. S. Lillard, J. F. Frank and S. E. Craven, University of Georgia, Food Science and Technology Department, Athens, GA 30602

This study was undertaken to determine if the viability of *Salmonella* cells affects their attachment to poultry skin.  $^3\text{H}$ -labeled *S. typhimurium* cells were inactivated by Gamma-irradiation (co-60) and by formaldehyde (5%). Their attachment rate was compared with live cells. The outer surfaces of broiler skin pieces were exposed to the cell suspension for 15 s, 5, 30 and 60 min and loosely attached cells were then rinsed off before counting. The  $\log_{10}$  of attached live cells for each respective inoculation time was 4.64, 5.07, 5.48 and 5.71 per  $\text{cm}^2$ . For Gamma-irradiated cells, counts were 4.90, 5.41, 5.90 and 6.09 and for formaldehyde-treated cells 3.90, 4.51, 5.13 and 5.49. Both Gamma-irradiated and formaldehyde-treated cells had a similar pattern and rate of attachment as live cells. Viability of *Salmonella* cells was not required for attachment to poultry skin.

#### EFFECT OF IRRADIATION ON SURVIVAL OF *SALMONELLA ENTERITIDIS* IN WHOLE EGGS AND LIQUID EGGS

Luvia Enid Serrano,\* Graduate Research Assistant and E. A. Murano, Departments of Animal Science and Microbiology, Immunology and Preventive Medicine, Iowa State University, Ames, IA 50011

In recent years *Salmonella enteritidis* has been implicated in several outbreaks of foodborne illness which were attributed to ingestion of contaminated eggs. Along with other processing treatments, irradiation has been considered as a method to eliminate this pathogen from eggs. The objectives of this study were to determine the ability of *S. enteritidis* to survive low-dose. Four animal isolates, one bovine, one from snake, and two from chicken were compared with ATCC 13076 in their survival to heat and/or irradiation. Whole shell eggs were internally inoculated with 1 ml of  $10^8$  cells/ml with one of each of the isolates and irradiated at 0, 0.25, 0.5 or 1.5 kGy. Results showed  $D_{10}$  values ranging from 0.16-0.24 kGy with isolates obtained from chicken and snake being more resistant than bovine isolates. Liquid egg samples were inoculated with 1 ml of  $10^8$  cell/ml with one of each of the isolates, heated at 50°C for 0, 20, 40 or 60 min prior to irradiation, and irradiated at 0, 0.25, 0.5 or 1.5 kGy. Non-heated (time 0), and irradiated samples were used as controls. Heat prior to irradiation at 0.25 kGy resulted in a 2  $\log_{10}$  reduction in the first 20 min for ATCC 13076, and a slight reduction in the other isolates at 60 min. Also, heating for 60 min prior to irradiation at 0.5 kGy, showed a 0.8  $\log_{10}$  reduction of the snake isolate and a 0.4  $\log_{10}$  reduction for the other isolates. Heating for 20 min followed by irradiation at 1.0 kGy resulted in a 2.0  $\log_{10}$  decrease in the bovine isolate, with snake and chicken isolates being reduced only after heating for 60 min followed by irradiation. Significant differences were also observed when the isolates were heated prior to irradiation at 1 kGy. The bovine isolate showed a 2  $\log_{10}$  reduction after 60 min. Finally, heating prior to irradiation at 1.5 kGy resulted in complete elimination of all isolates regardless of whether they were heated or not. Our results indicate that irradiation of whole eggs at doses as low as 0.6 kGy resulted in elimination of *S. enteritidis* and that mild heating prior to irradiation increases the effectiveness of that treatment.

#### MICROBIOLOGICAL EVALUATION OF REPROCESSED BROILER CARCASSES

Charles Powell,\* Graduate Student, G. Blank and R. Galopp, Food Science Department, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2

Canadian regulations require that carcasses with visible post-evisceration internal contamination be removed from the process line. Such carcasses are condemned after unaffected parts are salvaged by hot-boning. Contamination rates vary from less than 1% to more than 5% with a cost to the industry which may exceed \$4 million annually for each 1%. A study was conducted to determine the efficacy of reprocessing whole contaminated carcasses to a

state fit for entry to the chill tank. Over 300 inspection-passed and reprocessed carcasses were compared microbiologically using a new cup-rinse method to sample internal and external surfaces of interest. Contaminated carcasses were reprocessed manually off line and in a vent-down position by an immediate 5 s inside/outside (I/O) spray rinse followed by vacuuming and a 15 s I/O spray wash. Tap water at line pressure was used. Mean counts ( $\log_{10}/\text{ml}$ ) for inspection-passed and reprocessed carcasses were standard plate count (SPC) 3.99 and 3.55, coliforms 3.49 and 3.07, *Escherichia coli* 3.34 and 2.96 and *Staphylococcus* 2.42 and 1.53, respectively. Light and gross contaminated carcasses were distinguished visually and characterized by SPC, coliform and *E. coli* counts before any wash treatment. Following reprocessing, carcasses which had been grossly contaminated showed lower mean counts than control carcasses. Results showed that the reprocessing procedure successfully restored internally contaminated carcasses to the same or better microbiological status as inspection-passed carcasses.

#### CIDER COMPOSITION VERSUS HEAT RESISTANCE OF *ESCHERICHIA COLI* O157:H7

D. F. Splittstoesser,\* Professor, J. J. Churey and M. R. McLellan, Cornell University, NYS Agric. Exp. Station, Geneva, NY 14456

Apple cider has been a cause of foodborne illness due to the presence of *Escherichia coli* O157:H7. While pasteurization has been recommended as a means for eliminating a potential pathogen, most processors of cider are reluctant to adopt this treatment because of alterations in flavor. In this study, the minimal thermal treatment needed to destroy *E. coli* O157:H7 was determined in ciders in which percent sugar, pH, malic acid and concentration of preservatives were varied.  $D_{32}$  = about 12 min over a range of 11.8 to 16.5° Brix, a pH of 3.6 to 4.4, and 0.20 to 0.80% malic acid. Surface response plots showed that benzoic acid markedly reduced heat resistance: the presence of 0.1% resulted in an average  $D_{30}$  of 0.64 min.

#### *STAPHYLOCOCCUS INTERMEDIUS*: ETIOLOGIC ASSOCIATION WITH FOODBORNE INTOXICATION FROM BUTTER BLEND AND MARGARINE

Reginald E. Bennett,\* Microbiologist, Farukh M. Khambaty and Dhiren B. Shah, Food and Drug Administration, 200 C Street, S.W., Washington, DC 20204

Historically, *Staphylococcus aureus* has been recognized as a potential foodborne pathogen that is capable of proliferation and toxin formation in a wide variety of foods. More recently, other enterotoxigenic *Staphylococcus* spp. have been shown to be contaminants in foods. A food poisoning outbreak, involving over 265 cases, occurred in October 1991. *Staphylococcus intermedius* was isolated from patients, and from incriminated butter blend and margarine. To determine the etiology of this outbreak, the *S. intermedius* isolates were screened for enterotoxigenicity and the products were tested for preformed enterotoxins. All patient and product isolates produced staphylococcal enterotoxin A (SEA). Both untreated and untreated products contained SEA. The latter finding indicates that the products were contaminated prior to any heat treatment. The presence of viable enterotoxigenic *S. intermedius*, as a post-contaminant in the finished product may have contributed to the final quantity of preformed SEA in the incriminated products. Representative outbreak isolates (5, clinical; 10 food) were analyzed by pulsed-field gel electrophoresis (PFGE). These analyses, using four separate restriction endonucleases, provided definitive evidence that all isolates from nine different counties in California and Nevada were derived from a single strain. The PFGE pattern exhibited by these outbreak isolates is distinctly different from the patterns exhibited by a heterogeneous collection of seven *S. intermedius* strains of veterinary origin and five unrelated *S. aureus* strains. The data show a significant PFGE pattern diversity not only among members of different *Staphylococcus* spp., but also within members of the same species. The results indicate that PFGE is a valuable strain-specific discriminator for the epidemiological characterization of *S. intermedius*.

## IRRADIATION INACTIVATION OF *LISTERIA MONOCYTOGENES* AND *STAPHYLOCOCCUS AUREUS* IN GROUND BEEF AS AFFECTED BY FAT CONTENT AND TEMPERATURE

J. David Monk,\* Graduate Student, M. Rocelle S. Clavero, Larry R. Beuchat, Michael P. Doyle and Robert E. Brackett, Center for Food Safety & Quality Enhancement and Department of Food Science and Technology, University of Georgia, Griffin, GA 30223-1797

The influence of two levels of fat (11.1 to 13.9% [low-fat] and 27.1 to 27.9% [high-fat]) and temperature (frozen [-17 to -15°C] and refrigerated [3 to 5°C]) on gamma irradiation ( $^{60}\text{Co}$ ) inactivation of *Listeria monocytogenes* and *Staphylococcus aureus* in raw ground beef patties was investigated. Ground beef patties inoculated with stationary growth phase cells of five-strain mixtures of *L. monocytogenes* or *S. aureus* were treated with seven mean doses up to 2.062 or 2.147 kGy, respectively.  $D_{10}$  values ranged from 0.507 to 0.610 kGy and 0.435 to 0.453 kGy for *L. monocytogenes* or *S. aureus*, respectively. Neither the fat content of beef nor the temperature during irradiation treatment influenced inactivation rates of the two pathogens. Regression coefficients were high for all treatment conditions, the lowest being 0.984 and 0.990 for *L. monocytogenes* and *S. aureus*, respectively, in high-fat frozen beef. Based on the highest  $D_{10}$  value obtained, a dose of 2.50 kGy would theoretically kill 4.10  $\log_{10}$  *L. monocytogenes* and 5.12  $\log_{10}$  *S. aureus* per gram of ground beef.

## TRICHINOSIS OUTBREAK ASSOCIATED WITH SMOKED WILD BOAR MEAT, ONTARIO, CANADA

Barbara J. Marshall,\* Program Manager, Public Health Inspection Services and Sandy Isaacs, Wellington-Dufferin-Guelph Health Unit, 125 Delhi Street, Guelph, Ontario, Canada N1E 4J5

Satisfying the appetites of gourmets for fresh wild game and exotic meat is an exciting challenge for exclusive food establishments. However, prevention of the crisis of a Trichinosis outbreak in the population is a critical goal for public health and food safety officials.

Using a case study of an outbreak that occurred in Canada in January 1993; the presentation will describe the extensive Trichinosis investigation epidemiologically linked to the consumption of smoked wild boar meat. The investigation involved the collaborative efforts of the federal and provincial agricultural governments, the Ministry of Health and the medical community. The revision of the protocol for the inspection of wild boar carcasses at the slaughterhouse, and the adequate cooking, freezing or curing of wild boar meat are cited as preventive measures. This story was aired on national television (C.B.C.) on the "Health Show" in December 1993.

## ENTEROBACTERIACEAE FROM THE CHICKEN INTESTINE THAT USE PHOSPHATIDYL SERINE FOR GROWTH AND INHIBIT *SALMONELLA TYPHIMURIUM*

Stephen E. Craven, Microbiologist, U.S. Department of Agriculture, ARS, Russell Research Center, P. O. Box 5677, Athens, GA 30613

Several *Salmonella* serovars have been shown to utilize for growth lipids in the mucus layer of the mouse intestine, of which phosphatidyl serine (PS) was found to serve as the sole source of carbon, nitrogen and phosphorus (Krivan et al., 1992). Isolated chicken intestinal mucus supported the growth of *Salmonella typhimurium*. Generation times in chicken mucus and brain heart infusion were similar. Strains of Enterobacteriaceae that utilize PS for growth were isolated from the chicken intestinal tract. These strains competitively inhibited *in vitro* growth of *S. typhimurium* in chicken mucus. When given by oral gavage to day-of-hatch chicks, a composite (6 to 12 isolates) of these strains reduced cecal colonization of analidixic-acid resistant strain of *S. typhimurium* administered 4 to 24 h later. Bacterial strains that utilize PS may be useful components of a defined competitive exclusion culture effective against salmonellae.

## CHARACTERIZATION OF PYOCYANINE PRODUCED BY *PSEUDOMONAS AERUGINOSA*

Nassim H. Nabbut, Ph.D., Professor of Microbiology, Faculty of Medicine, American University of Beirut Medical Center, Beirut, Lebanon

*Pseudomonas aeruginosa* is widely distributed in soil, water, sewage, vegetables and the hospital environment. Currently, it is one of the most

important opportunistic pathogens responsible for infections in hospitalized patients. It produces pyocyanine, a blue-green phenazine pigment known as 5-methyl-hydroxyphenazine.

Different culture media and analytical methods have been developed for the production and quantitative as well as qualitative determination of pyocyanine. In our work, we have used a modification of Frank and De Moss medium for the production of this pigment. The effects of a range of pH values of the medium, mineral sources, oxidizing agents, temperature and time of incubation and different amino acids on pyocyanine production were determined.

Pyocyanine was extracted from the culture filtrates of *P. aeruginosa* and grown in modified Frank and DeMoss medium. It was then purified by thin layer chromatography followed by distillation of the chloroform extracted pigment. Preliminary testing of the antibacterial effect of purified pyocyanine showed that it is inhibitory to *Staphylococcus aureus*, *Bacillus cereus*, *Shigella flexneri* and *Escherichia coli*.

Work is in progress to test the antibacterial effect of pyocyanine using a wide spectrum of gram-positive and gram-negative bacteria.

## EFFECTS OF IONIZING RADIATION AND ANAEROBIC REFRIGERATED STORAGE ON INDIGENOUS MICROFLORA, *SALMONELLA* AND *CLOSTRIDIUM BOTULINUM* TYPES A AND B IN MECHANICALLY-DEBONED CHICKEN

Donald W. Thayer,\* Research Leader, G. Boyd and C. N. Huhtanen, Eastern Regional Research Center, 600 E. Mermaid Lane, Philadelphia, PA 19118

We tested the concept that *Clostridium botulinum* might produce toxin beyond signs of spoilage using vacuum-canned, commercial, mechanically-deboned chicken meat challenged with *C. botulinum*, types A and B, and *Salmonella enteritidis*. Irradiated (0, 1.5 and 3.0 kGy) samples were stored at 5°C for 0, 2 and 4 weeks. As expected, the highly radiation-resistant endospores of types A and B *C. botulinum* survived the treatment. No refrigerated sample was toxic; however, all inoculated samples became toxic when temperature abused. *Salmonella enteritidis* in non-irradiated samples decreased during storage from  $\log$  3.86 to 2.58. It was countable only in samples irradiated to 1.5 kGy at 0 weeks and was not detected by enrichment culture in samples irradiated to 3.0 kGy. Populations of aerobic or facultative mesophiles increased during 4 weeks of refrigerated storage from  $\log$  6.54 to 8.25, 4.03 to 8.14, and 2.84 to 5.23 in the samples irradiated to 0, 1.5 and 3.0 kGy, respectively. Based on taxonomic analyses of 245 isolates, the microbial populations of samples shifted as a function of both radiation dose and storage time from predominantly gram-negative in non-irradiated samples to gram-positive streptococci in samples irradiated to 3.0 kGy and stored for 4 weeks. Significant populations of spoilage organisms survived even the 3.0 kGy treatment.

## EFFICACY OF CULTURED WHEY OF ANTAGONISTIC MICROORGANISMS TO INHIBIT PSYCHROTROPHIC PATHOGENS IN REFRIGERATED, COOKED BEEF AND POULTRY

Y.-Y. Hao,\* Postdoc. Research Assistant, R. E. Brackett and M. P. Doyle, Center for Food Safety and Quality Enhancement, Department of Food Science and Technology, University of Georgia, Griffin, GA 30223

The efficacy of using cultured whey prepared from seven antagonistic bacteria to inhibit growth of *Aeromonas hydrophila* and *Listeria monocytogenes*, in refrigerated, cooked beef and poultry was studied. Cultured whey (CW) was obtained by centrifuging milk that was inoculated with one of 7 antagonistic bacteria and incubated for 24 h. Cultured whey was evenly distributed on the surface of cooked beef or chicken breast after the sample was inoculated with either low ( $10^1$  CFU/g) or high ( $10^8$  CFU/g) populations of *A. hydrophila* and *L. monocytogenes*. Samples were then incubated at 5° or 15°C. Microbial populations were determined periodically using starch ampicillin agar (*A. hydrophila*) or modified Oxford agar (*L. monocytogenes*). Cultured whey differed significantly in ability to inhibit growth of pathogens on different muscle tissue samples, and the effects were most obvious at the end of storage. In general, *Lactococcus lactis* was effective at inhibiting growth of both high and low initial populations of *L. monocytogenes* on chicken breast at 15°C while *Lactobacillus sake* and *Carnobacterium piscicola* were effective at reducing growth of low initial population of LM. After 1 week of incubation at 15°C, the difference in populations of *L. monocytogenes* between chicken treated with CW and no treatment was  $>2 \log_{10}$  CFU/g when initial population was  $10^8$ , but was about  $1 \log_{10}$  CFU/g when the initial population of AH on beef at 5 and 15°C, respectively. After

14 (5°C) or 7 (15°C) days storage, AH populations on samples treated with CW differed from untreated beef by 3.5 or 1.5 log<sub>10</sub> CFU/g, respectively.

## STAINLESS STEELS FOR DAIRY AND FOOD EQUIPMENT SYMPOSIUM

### UTILIZING STAINLESS STEELS IN THE FOOD AND DAIRY INDUSTRIES

Peter Elliott, Ph.D., C. Eng., President, P.E. Corrosion and Materials Consultancy, Inc., 29 State Highway 34 North, Suite 203, Colts Neck, NJ 07722

The widespread use of stainless steels in food and dairy produce handling can be directly related to the benefits afforded by these materials of construction, especially: corrosion resistance; ease of fabrication; strength and ductility over a range of temperatures; toughness and resistance to abrasion and erosion; good surface finish; attractive heat transfer properties; and, ease of cleaning.

With continued stringencies for health and hygienic standards, it is anticipated that traditional stainless steels (types 304 and 316), and more recently developed alloys offering better corrosion resistance will continue to dominate this industry. The specially developed corrosion resistant alloys (CRA's) include the 6% molybdenum alloys, e.g., Al-6XN, 254 SMO and the duplex austenitic/ferritic steels, e.g., 2205, Ferralium 255, 7-Mo PLUS. These CRA's are finding application under conditions that induce pitting and stress corrosion cracking in the more traditional alloys, e.g., in chloride-containing solutions, steam cleaning, heat exchangers, etc.

Notwithstanding the excellent properties of the stainless steels there are occasions where poor design, incorrect or inadequate maintenance, etc., can seriously impair the surface properties and on occasion lead to premature failure. Put simply, premature failure can often be considered a management issue, as will be demonstrated. Many problems occur because of a breakdown in communication channels or because of the "human error" factor.

This talk will address these issues and will indicate methods for minimizing the risk of surface deterioration, and for maximizing the longevity of plant materials.

### FABRICATION AND APPLICATION OF STAINLESS STEEL EQUIPMENT FOR SANITARY APPLICATIONS

Vincent Mills, Evergreen Packaging Equipment, 2400 Sixth Street, SW, P.O. Box 3000, Cedar Rapids, IA 52406-3004

The Food Processing Industry calls for stainless steel equipment that is capable of being thoroughly cleaned and sanitized. The presentation reviews equipment design for cleaning, either manually or mechanically, with mechanical cleaning as the preferred method. Moist heat (steam under pressure) is often used to either sanitize or sterilize the equipment. Equipment must meet ASME standards as well as the 3-A Sanitary Standards, and must comply with OSHA requirements. The melding of these three standards is essential for equipment to be utilized in the food processing industry.

### ORBITAL WELDING OF STAINLESS STEEL TUBING FOR FOOD AND DAIRY APPLICATIONS

Barbara Henon, ARC Machines, Inc., 10280 Glenoaks Blvd., Pacoima, CA 91331

Orbital welding is the preferred method of joining stainless steel tubing for piping systems in the food and dairy industries. The smooth inner weld bead produced by orbital welding provides a surface that is easily cleanable which is particularly important for piping systems designed to be cleaned or sterilized in place (CIP/SIP). Visual weld acceptance criteria include full penetration with an even uniform weld bead around the entire circumference that is neither excessively concave nor convex and with minimal discoloration due to oxidation. Quality control is the owner's responsibility. Weld parameters, regulated by the welding power supply, are based on tube dimensions. For example, weld current (or amperage) is proportional to the tubing wall thickness. The RPM of the weld head rotor is based on electrode surface travel speed so that the larger the tube, the slower the RPM. Weld parameter selection for single pass fusion butt welds of thin-walled tubing up to 4 inches in diameter, as well as the use of step rotation or tube-to-fitting welds will be described. Heat-to-heat variations in the weldability of 316 L stainless steel will be discussed in terms of trace elements with emphasis

placed on the sulfur content. Fabrication practices including tube-end preparation, purging and tacking procedures that promote optimum cleanliness and service life of welded piping systems will be discussed. Considerations for the welding of alternative materials such as super austenitic stainless steels and nickel alloys will be examined.

### THE EFFECT OF SURFACE FINISH ON THE BEHAVIOR OF STAINLESS STEEL IN FOOD AND DAIRY SCIENCE

Arthur H. Tuthill, P.E., Tuthill Associates, Inc., P. O. Box 204, 2903 Wakefield Drive, Blacksburg, VA 24060

The food industry is concerned with purity of product and cleanliness of the process equipment it uses. The author is frequently consulted on how to passivate stainless steel. Invariably what the client really means is how to clean stainless steel surfaces. The nature of the surface of stainless steel and the effect of welding, heat tint, grinding, blasting, passivation, pickling, and electropolishing on surface condition will be reviewed with special attention to inherent cleanliness of the surface in contact with food and dairy products.

### HYGIENE AND OTHER HEALTH AND SAFETY ASPECTS OF STAINLESS STEEL IN FOODHANDLING AND PROCESSING PLANTS

J. H. Lilly, Nickel Development Institute, 214 King Street West, Suite 510, Toronto, Ontario, Canada M5H 3S6

Concern about the purity of food beverages vis-a-vis human health, places emphasis on the "health aspects" of stainless steel in several major markets. In this paper, the results of relevant researches are presented. In relation to bacterial contamination, it is shown that after simulated wear, contamination and washing procedures, stainless steel sinks retain up to 100-times fewer bacteria than sinks made in competitive materials.

In relation to metal contamination, it is shown that release of nickel into even the most aggressive foods cooked in stainless steel pans is small in comparison with the level of nickel found in typical human diets. Contrary to recent media suggestions that stainless steel pots should be avoided because they contribute toxic amounts of nickel to food cooked in them, scientific studies have confirmed that nickel pickup is generally undetectable. Even the greatest pickup in brand-new pots is only a modest portion of the nickel intake in normal daily diets and is insignificant compared to the quantities of ingested nickel required to induce allergic reactions, even in persons already sensitized to nickel.

Stainless steel pots are effectively irrelevant to daily intake of ingested nickel and remain the material of choice for modern cookware and for the hygienic preparation and handling of food.

### MEAT QUALITY AND SAFETY: EFFECTS OF PRODUCTION AND PROCESSING ON THE MICROBIAL QUALITY OF MEAT

*Sponsored by the Ontario Food Protection Association*

### INNOVATIONS IN AUSTRALIAN MEAT PROCESSING PRACTICES AND SLAUGHTER OPERATIONS: THEIR IMPACT ON MICROBIAL STATUS

Barry Shay, CSIRO Australia, Meat Safety Laboratory, Group Coordinator, Meat Safety and Preservation, P.O. Box 12, Cannon Hill, Brisbane, Queensland 4170 Australia

Whilst Australia is not the largest global producer of beef, it is the world's largest beef exporter. As the industry is highly aware of the need to produce meat of a high microbiological standard, it has an ongoing interest in the development and utilization of new and existing processing technologies that will maintain and improve upon the high standards that it currently achieves. Like the USA, the industry's R & D body, the Meat Research Corporation, has funded CSIRO in conjunction with Australia's regulatory authority (Australian Quarantine Inspection Service) to carry out a microbiological survey on beef and sheep meat produced out of both export and domestic slaughterhouses. Whilst similar in many ways to the U.S. beef baseline survey, the project has a somewhat broader scope in that it will generate microbiological information on the impact of new processing technologies and procedures when they are carried out under commercial conditions. The new processing technologies and procedures which are of current interest to the Australian meat industry include hot boning, spray chilling, automated slaughter (Fututech), decontamination (hot water and organic acid treatments) and the substitution of company quality assurance



# CALL FOR PAPERS

## IAMFES

82<sup>nd</sup> Annual Meeting July 30-August 2, 1995  
Pittsburgh, Pennsylvania

### Instructions to Prepare Abstracts

#### Procedure

- Use the printed Abstract form that appears on the other side of this page.
- Type in the title, CAPITALIZE the first letter of the first word and proper nouns.
- List the names of authors and institution(s). Capitalize first letters and initials.
- Give the name, title, mailing address and the office telephone number of the author who will present the paper.
- If the paper is to be presented by a student entered in the Developing Scientist Awards Competitions, check the box to indicate this and have the form signed by your Major Professor or Department Head.
- Check the most appropriate box to indicate the general subject area of the paper. Indicate subject if checking "other."
- Type the abstract double-spaced, in the space provided on the abstract form.

Mail two copies of the abstract before December 15, 1994 to:

Steven K. Halstead, CAE  
Executive Manager, IAMFES  
6200 Aurora Avenue  
Suite 200W  
Des Moines, IA 50322-2838

Enclose two stamped, self-addressed postcards. Two cards must be included with each abstract that is submitted. One will be returned to acknowledge receipt of the abstract and the other to notify the presenter of the time the paper is to be presented.

#### Content of the Abstract

The abstract should describe briefly: (a) the problem studied, (b) methods applied, (c) essential results, and (d) conclusions.

#### Presentations Format:

Papers may be presented orally or by poster format at the discretion of the Program Committee. Oral presentations will be scheduled so a speaker has a maximum of 15 minutes, including a 2-4 minute discussion. Carousel projectors for 35 mm slides will be available.

Overhead projectors are not to be used and none will be available.

#### Subject Matter for Papers

Papers should report the results of applied research on: food, dairy and environmental sanitation; foodborne pathogens; food and dairy microbiology; food and dairy engineering; food and dairy chemistry; food additives and residues; food and dairy technology; food service and food administration; quality assurance/control; mastitis; environmental health; waste management and water quality.

#### Developing Scientist Awards Competitions

The **Oral Competition** is open to GRADUATE students enrolled at accredited universities or colleges whose research deals with problems related to environmental, food and/or dairy sanitation, protection and safety. Candidates cannot have graduated more than one (1) year prior to the deadline for submitting abstracts.

This year the Oral Competition will be limited to up to ten finalists and awards will be given to the top three presenters. The papers should be approximately fifteen (15) minutes, including a 2-4 minute discussion.

The **Poster Competition** is open to UNDERGRADUATE and GRADUATE students enrolled at accredited universities or colleges whose research deals with problems related to environmental, food and/or dairy sanitation, protection and safety. Candidates cannot have graduated more than one (1) year prior to the deadline for submitting abstracts.

Up to 10 finalists will be selected for the Poster Competition. The presentation must be mounted on an 8' by 4' display board (provided at the meeting) for the entire duration of the Poster Session at the Annual Meeting. The presenter must be present at their poster for a specific time, approximately two hours during the session. (For more information on the Developing Scientist Awards Competitions, see the following green pages.)

All winners are presented and honored at the annual Awards Banquet. The finalists will receive complimentary tickets and are expected to be present at the Banquet.

#### Additional Abstract Forms

Extra copies of the abstract forms may be obtained from Steven K. Halstead, Executive Manager, or you may photocopy this one.

#### Membership in IAMFES

Membership in IAMFES is NOT a requirement for presenting a paper at the IAMFES Annual Meeting.

**IAMFES Abstract Form**  
**DEADLINE: DECEMBER 15, 1994**

Title of Paper \_\_\_\_\_  
\_\_\_\_\_

Authors \_\_\_\_\_  
\_\_\_\_\_

Name and Title of Presenter \_\_\_\_\_  
\_\_\_\_\_

Institution and Address of Presenter \_\_\_\_\_  
\_\_\_\_\_

Office Phone Number (\_\_\_\_) \_\_\_\_ - \_\_\_\_\_

Developing Scientist Awards Competition  Yes  Oral  Poster

Major Professor/Department Head approval (signature and date) \_\_\_\_\_

**General Subject Area**

- |  |                                       |
|--|---------------------------------------|
| <input type="checkbox"/> Quality Assurance/Control | <input type="checkbox"/> Food Service |
| <input type="checkbox"/> Food Microbiology         | <input type="checkbox"/> Sanitation   |
| <input type="checkbox"/> Dairy Microbiology        | <input type="checkbox"/> Food Safety  |
| <input type="checkbox"/> Waste Management          | <input type="checkbox"/> Processing   |
| <input type="checkbox"/> Lab Methods               | <input type="checkbox"/> Epidemiology |
| <input type="checkbox"/> Foodborne Pathogens       | <input type="checkbox"/> Other _____  |
| <input type="checkbox"/> Chemical Residues         | _____                                 |
| <input type="checkbox"/> Environmental Health      | _____                                 |

**Check the format you prefer.**

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| <input type="checkbox"/> Oral          | <input type="checkbox"/> Poster        |
| <input type="checkbox"/> Video Theater | <input type="checkbox"/> No Preference |

**Please TYPE Abstract, DOUBLE-SPACED, in the space provided here.**

**Selected presentations, with permission, will be recorded (audio or visual).**

I authorize IAMFES to record my presentation.

Signature \_\_\_\_\_ Date: \_\_\_\_\_

I do not wish to be recorded.

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**Announcement:**  
**Developing Scientist Awards Competitions**  
**(Supported by Sustaining Members)**

IAMFES is pleased to announce continued extension of its program to encourage and recognize the work of students in the field of food safety research. In addition to the Oral Developing Scientist Awards Competition, IAMFES will again offer a Poster Presentation Award Competition.

**Purpose**

1. To encourage graduate and undergraduate students to present their original research at the IAMFES meeting.
2. To foster professionalism in students through contact with peers and professional members of IAMFES.
3. To encourage participation by students in IAMFES and its annual meeting.

**Developing Scientist Oral Competition:**

The Oral Competition is open to GRADUATE students enrolled in M.S. or Ph.D. programs at accredited universities or colleges whose research deals with problems related to environmental, food and/or dairy sanitation, protection and safety. Candidates cannot have graduated more than one (1) year prior to the deadline for submitting abstracts.

This year the Oral Competition will be limited to ten (10) finalists and awards will be given to the top three (3) presenters. The papers should be approximately fifteen (15) minutes long including a two to four (2-4) minute discussion.

**Awards:** First Place: \$500 and a Plaque; Second Place: \$300 and a certificate of merit; Third Place: \$100 and a certificate of merit. All of the winners will receive a one-year membership including both *Dairy, Food and Environmental Sanitation* and the *Journal of Food Protection*.

**Developing Scientist Poster Competition:**

The Poster Competition is open to UNDERGRADUATE and GRADUATE students enrolled at accredited universities or colleges whose research deals with problems related to environmental, food and/or dairy sanitation, protection and safety. Candidates cannot have graduated more than one (1) year prior to the deadline for submitting abstracts.

Up to ten (10) finalists will be selected for the Poster Competition. The presentation must be mounted on an 8' by 4' display board (provided at the meeting) for the entire duration of the Poster Session at the Annual Meeting. The presenter must be present at his/her poster for a specific time, approximately two hours, during the session.

**Awards:** First Place: \$500 and a Plaque; Second Place: \$300 and a certificate of merit; Third Place: \$100 and a certificate of merit. All of the winners will receive a one-year membership including both *Dairy, Food and Environmental Sanitation* and the *Journal of Food Protection*.

Instructions to Developing Scientist Awards Competitions Entrants (Oral and Poster):

**\*Note:** Abstracts must be submitted to the IAMFES office no later than December 15, 1994. No forms will be sent to entrants. Enclose two self-addressed, stamped postcards with your submitted abstracts.

1. One original and four copies of an abstract of the paper must be submitted on the green abstract form found in the September or October issues of the IAMFES journals. Indicate on the abstract form whether the presentation is submitted for the Oral or Poster Competition.
2. The presentation and the student must be recommended and approved for the Competition by his/her Major Professor or Department Head, who must sign the Abstract.
3. The work must represent original research done by the student and must be presented by the student.
4. Each student may enter only one (1) paper in either the Oral or Poster Competition.
5. All students will receive confirmation of acceptance of their presentations along with guidelines for preparing their Oral or Poster Presentations.
6. All Students with accepted abstracts will receive a complimentary membership which includes their choice of *Dairy, Food and Environmental Sanitation* or the *Journal of Food Protection*.
7. Winners are announced at the Annual Awards Banquet. The finalists for the Oral Competition and the Poster Competition will receive complimentary tickets and are expected to be present at the Banquet.

## **Judging Criteria for Developing Scientist Awards Competitions**

### **Judging**

The abstracts and presentations will be evaluated by an independent panel of judges. Selection of up to ten (10) finalists for both the Oral and Poster Competitions will be based on evaluations of the abstracts and the scientific quality of the work (see judging criteria). All entrants in the Developing Scientist Awards Competitions will be advised of the judges' decisions by March 31, 1995.

Only the ten (10) finalists in each category will be judged at the Annual Meeting and will be eligible for the final awards. All other entrants who submitted papers accepted by the IAMFES Program Committee will be expected to present their papers/posters as part of the regular Annual Meeting program, but their presentations will not be judged and they will not be eligible for awards.

### **Judging Criteria**

#### **ABSTRACT**

Clarity, comprehensiveness, conciseness;

#### **SCIENTIFIC QUALITY**

Adequacy of experimental design;

Extent objectives were met;

Difficulty of research, depth;

Validity of conclusions based upon data;

Technical merit, contribution to science;

#### **ORAL PRESENTATION or POSTER PRESENTATION**

Organization: clarity of introduction, objectives, methods, results and conclusions;

Quality of visuals;

Quality and poise of presentation and in answering questions.

**\* NOTE: Your abstract must be submitted to the IAMFES office no later than December 15, 1994. No forms will be sent to entrants. Enclose two self-addressed, stamped postcards with your original abstract and four copies.**

systems for government meat inspection. The microbiological results of laboratory and commercial trials will be discussed.

#### **VTEC/CYTOTOXIGENIC *ESCHERICHIA COLI*: THE DAIRY FARM AS A MODEL FOR ANIMAL-HUMAN TRANSMISSION**

Robert C. Clarke,\* Director, J. B. Wilson, S. Renwick, K. Rahn, R. P. Johnson, D. Alves, M. A. Karmali, H. Lior, S. A. McEwen and J. Spika, Agriculture and Agri-Food Canada, Health of Animals Laboratory/Guelph, 110 Stone Road West, Guelph, Ontario, Canada

Our understanding of the transmission of VTEC from animals to humans has largely been acquired through the investigation of outbreaks of severe clinical disease in humans. We have investigated an occupationally exposed group (dairy farm families) to examine factors that influence the transmission of VTEC in a non-outbreak setting. Fecal samples from 336 humans, 592 calves and 886 cows on 80 dairy farms in Ontario were tested for VT *Escherichia coli* (VTEC) using a Vero cell assay, and a PCR procedure. Persons were questioned regarding episodes of enteric illness and risk factors for VTEC infection. Farms having a VTEC-infected family member or an *E. coli* O157:H7-infected animal were revisited one and three months later. On the initial visit, 21 family members (8%) on 16 grams (21%) were positive for VTEC. The VTEC infection was not associated with diarrheal illness in any of the humans at the time of sampling and most of the positive VTEC cultures from humans occurred in late summer-early fall. Three persons and six families were VTEC-positive on two occasions. VTEC serotypes identified in humans were 05:NM, 07:H4, 080:NM, 091:H14, 0103:H2, 0132:NM, 07:H21, 07:H25 and O157:H7. All 80 herds, 36% of cows and 57% of calves were VTEC-positive on the first visit. Seven animals (0.45%) on four farms (5%) were positive for *E. coli* O157:H7 on the first visit. No animal was positive for *E. coli* O157:H7 on more than one occasion, however, two farms were positive for *E. coli* O157:H7 on two visits. The peak of *E. coli* O157:H7 infection in cattle was between June and August. Our study indicates that transient carriage of VTEC, including serotype O157:H7, occurs in clinically normal cattle and humans in the farm setting.

#### **FSIS NATIONWIDE BEEF MICROBIOLOGICAL BASELINE DATA COLLECTION PROGRAM: SURVEY OF STEERS AND HEIFERS**

Ann Marie McNamara, U.S. Department of Agriculture, FSIS, Director, Microbiology Division, 300 12th Street, S.W., Washington, DC 20250

From October 1992 through September 1993, tissue samples representing approximately 2,100 steer or heifer carcasses were collected from establishments operating under federal inspection. Samples were collected to estimate the prevalence and levels of bacteria of public health concern on steer and heifer carcasses as currently produced. The establishments included in the program are responsible for approximately 99% of domestic origin steer and heifer production. The tissue samples were analyzed for the presence of those bacteria most often associated with human illness as determined by foodborne illness reports, other pathogens of interest because of the severity of human illness they produce, and certain bacteria, or groups of bacteria, thought to be of value as indicators of general hygiene or process control. *Clostridium perfringens* was recovered from 2.6% of 2,079 carcasses. *Staphylococcus aureus* was recovered from 4.2% of 2,089 carcasses. *Listeria monocytogenes* was recovered from 4.1% of 2,089 carcasses. *Campylobacter jejuni/coli* was recovered from 4.0% of 2,064 carcasses. *Escherichia coli* O157:H7 was recovered from 0.2% of 2,081 carcasses and *Salmonella* was recovered from 1.0% of 2,089 carcasses. Of the samples tested, 93.1% had aerobic plate counts (APC @35°C) of 10,000 or fewer colony forming units (CFU) per cm<sup>2</sup>, 96.4% contained 100 or fewer coliforms per cm<sup>2</sup>, and 95.9% contained 10 or fewer *Escherichia coli* (Biotype I) per cm<sup>2</sup>. Biotype I, *E. coli* are generally considered non-pathogenic. The APC levels are in agreement with those reported as normal in 1985 by the National Academy of Sciences for freshly dressed U.S. beef carcasses.

#### **CANADIAN MEAT INDUSTRY PERSPECTIVES ON HOW TO ADDRESS FOODBORNE ILLNESS**

Dr. Anne MacKenzie, Director General, Food Inspection Directorate, 59 Camelot Drive, Nepean, Ontario, Canada K1A 0Y9

The Food Safety Enhancement Program (FSEP) is the name of Agriculture and Agri-Food Canada's (AAFC) program to encourage the develop-

ment of Hazard Analysis Critical Control Point-based systems in all federally registered agri-food establishments. The core of the program is the internationally recognized Hazard Analysis Critical Control Point (HAACP) system. The program is being introduced to more than 1600 federally registered plants across the four major industry sectors regulated by AAFC: meat and poultry, dairy, fruit and vegetable, and egg products. To successfully implement HACCP, a real partnership between the Canadian agri-food industry and government is essential. The success of the program depends on consultation in all aspects of program development: training, communications and collaborative work to substantiate the science-base of the HACCP program and its practicality.

A major government/industry effort is underway to develop HACCP generic models through on-site pilot projects and expert committees. Generic models are available to industry as tools for the development of customized in-plant HACCP systems. In addition, a hazard reference database regarding incoming materials and processing steps has been developed to facilitate one of the key steps of HACCP, hazard identification.

The preliminary results of HACCP implementation in Canada are very encouraging. Reduced product rework and wastage, less returned product, better internal communication with inspection services are some benefits that have been experienced by industry. The HACCP implementation in Canada will help position the agri-food industry to meet upcoming international trade expectations.

#### **HACCP FROM PEN TO PLATE**

W. Ronald Osborne, Caravelle Foods, Vice President, Quality Assurance, 235 Nuggett Court, Brampton, Ontario, Canada L6T 5H4

Little is done during the manufacture of fresh or frozen hamburger patties to change the microbial quality of this product from that of the raw material. Consequently, the inputs for patty production are critical. Descriptions of these inputs will be given followed by a discussion of the importance of the application of the HACCP principles throughout the "hamburger system." Emphasis will be given on how to obtain high quality raw materials. Some illustrations of critical control points and the related standard operating procedures during the slaughter and boning operations will be used. This will be followed by a discussion on the use of HACCP and handling practices during patty production, distribution and at the restaurant. The importance of cooperation between the raw material supplier and patty processor to achieve common food safety goals will also be discussed.

#### **SCIENTIFIC POSTER SESSION**

##### **SUMMARY OF STANDARD PLATE COUNTS OF PLANT OBTAINED CHOCOLATE MILK AND DRINKS AFTER 14 DAYS AT 7.2° C (45° F)**

S. E. Barnard,\* Professor of Food Science, and R. N. Bicher, The Pennsylvania State University, University Park, PA 16802

One-hundred-and-fifty-five samples of chocolate flavored milk or drinks in retail packages were obtained from milk plants in Pennsylvania during calendar year 1993. Following incubation for up to 14 days at 7.2 C (45 F), 128 (83%) of these samples were judged by one or more trained dairy product evaluators to be of acceptable flavor with flavor scores of 6 or above. The remaining 27 (17%) samples were judged as spoiled with a score of 0 by ADSA scoring method. Microbiological analysis of the samples showed 80 (52%) of the samples had coliform counts of <1 per ml. Thirteen (8.4%) of the samples had SPCs of from 5.1 x 10<sup>2</sup> to 6.4 x 10<sup>5</sup> CFU per ml and acceptable flavor. The interesting results were that the 142 (92%) samples had SPC'S OF 1.4 to 590 x 10<sup>6</sup> CFU per ml., and 115 of these samples has acceptable flavor. Heat treatment of up to 12 samples each was done in submerged test tubes at 66° C or 80° C, the latter for both 15 and 30 min. Heat treatment followed by the holding at 45° F for 14 days and failed to destroy the microorganisms present. Previously, microbiological determinations of combinations of chocolate flavor powder, sterile water, sugar and milk failed to reveal the source of contamination. Additional studies have been undertaken.

## RAPID COLORIMETRIC METHOD FOR ESTIMATION OF RANCIDITY IN DAIRY PRODUCTS

Troy A. Bauer,\* Student-Research Assistant, Purnendu C. Vasavada, University of Wisconsin, River Falls Animal and Food Science Dept., UWRF-River Falls, WI 54022

A colorimetric method based on a gel-in-tube instant-chemistry (GITIC) involving reaction between a portion of milk fat and the melted gel reagent containing reactive matrix was evaluated for rapid determination of the free fatty acids in the milk, butter and cheese samples. A standard curve using oleic acid was used to estimate the Acid Degree Value (ADV) of samples and comparison was made with the ADV obtained by the standard method. The ADV's for milk samples by the GITIC and the standard methods ranged from 0.46-2.52 and 0.62-2.15, respectively. For butter and cheese samples, the ADV's obtained by the GITIC method were normally higher than with the standard method. The GITIC method required 15 to 30 min compared to 2-3 h for the standard ADV method. Refinements in fat extraction process may provide simple and rapid methods for estimation of rancidity in dairy products.

## SURVIVAL OF BRUCELLA ABORTUS IN THE MEXICAN WHITE SOFT CHEESE PROCESSING

Martha E. Diaz Cinco,\* Acedo F., Evelia and Leon D., and Alma B., Centro De Investigacion En Alimentacion Y Desarrollo, A.C. APDO, Postal no. 1735, Hermosillo Sonora, Mexico 83000

Brucellosis is an endemic disease in Mexico. The majority of cheese is consumed without pasteurization. The objective of this study was to determine the survival of *Brucella abortus* in Mexican white soft cheese manufactured in our laboratory. Prior to adding the renin, the milk was inoculated with  $3 \times 10^9$  CFU/ml of *B. abortus*: 3 cheeses were elaborated. Chemical analysis including fat, protein, salt, humidity, water activity ( $a_w$ ) and pH were determined to correlate with the recovery of *Brucella*. The cheese was held for 21 days at 5°C. A triplicate sample was taken for the bacteria analysis and it was incubated at 37°C, 8% CO<sub>2</sub> and every 72 h a triplicate subsample was evaluated as well.

Two methods (A and B) were evaluated to recover the organisms. Method A had 55% recovery of *Brucella abortus* in both enrichment mediums, while method B showed 46% recovery. *Brucella* agar had the best recovery followed by potato infusion agar, BHI agar, tripticase soy agar and plate count agar, in that order. The chemical parameters which had influence on recovery were fat and salt (Tukey's test,  $p < 0.05$ ). Protein, humidity, pH and  $a_w$  had no significant influence. The cheese processing inhibited *Brucella* but it was not eliminated.

## S-VALUE AND EPIFLUORESCENCE DETERMINATION OF BACTERIAL ATTACHMENT ON THE CLEANING BRUSH OF AN AUTOMATIC MILKING SYSTEM

Chen-Jang Liu,\* Student and D. C. Westhoff, Food Science Program, Department of Animal Sciences, University of Maryland, College Park, MD 20742

A method for S-value determination was developed to examine bacterial attachment onto an udder/teat cleaning brush of a prototype automated milking system. Twelve isolates from the brush bristles had S-values ranging from -0.06 to 2.24. *Bacillus sphaericus*, *Acinetobacter calcoaceticus*, *Pseudomonas putida* and *Acinetobacter calcoaceticus* with S-values of 2.24, 1.94, 0.68 and 0.64, respectively, demonstrated the strongest attachment. Negative or small S-values represent unstable or weak attachment. Strong or stable attachment (higher S-value) also showed more acceptable coefficients of variation (e.g., C.V. = 12%). A modified procedure for epifluorescent microscopic examination confirmed that the bacteria strains with the higher S-values not only attached firmly but also attached to the bristle faster.

## ENRICHMENT PROCEDURES AFFECTING THE SENSITIVITY OF THE EHEC™ ELISA SYSTEM

Scott R. Jeffrey,\* Research Scientist I, Rebecca J. Durham and Barbara Robinson, Organon Teknika Corporation, 100 Akzo Avenue, Durham, NC 27712

Ground beef contaminated with *Escherichia coli* O157:H7 is the food most often implicated in outbreaks of hemorrhagic colitis associated with

Hemolytic Uremic Syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP). EHEC-Tek employs a monoclonal antibody (4E8C12) which recognizes *E. coli* O157:H7 and other verotoxigenic strains. When bile salts are present, the antigen recognized by 4E8C12 is expressed not only in *E. coli* O157:H7, but in several other verotoxigenic strains of *E. coli*. Removal of bile salts results in detection by ELISA of only *E. coli* O157:H7.

This study was undertaken to determine what effects agitation and the exclusion of bile salts in the enrichment have on sensitivity of the ELISA. In multiple spiked ground beef samples which were confirmed by culture, the absence of bile salts from the enrichment resulted in an increase in OD values of samples spiked at the same level and approximately one log greater sensitivity when samples were spiked at multiple levels. Agitation during enrichment is also required for maximum sensitivity.

## RAPID DETECTION OF ENTEROTOXIGENIC CLOSTRIDIUM PERFRINGENS IN BEEF USING AN ALKALINE PHOSPHATASE MICROCOLONY TECHNIQUE

Luis A. Baez,\* Research Associate and Vijay K. Juneja, U.S. Department of Agriculture, ARS, ERRC, 600 E. Mermaid Lane, Philadelphia, PA 19118

A rapid technique was developed to detect and specifically identify enterotoxigenic *Clostridium perfringens* strains in beef by combining a microcolony technique with an indirect alkaline phosphatase conjugated antibody. Ground beef samples containing enterotoxigenic *C. perfringens* cells were diluted in phosphate buffer saline containing 0.1% Tween 80 and filtered through 0.2 µm cellulose nitrate membranes. Thereafter, the membranes were transferred onto the surface of a solid Duncan and Strong medium and incubated at 37°C for 2 to 18 h. The membranes were sequentially overlaid with *C. perfringens* anti-enterotoxin and an alkaline phosphatase conjugated antibody resulting in a chromogenic reaction that enabled detection and colony count. The results were in agreement with conventional plate count on Tryptose-sulfite-cycloserine agar. The technique is sensitive enough to detect as low as log<sub>10</sub> 2.0 CFU/g as early as 6 h.

## DEVELOPMENT OF TWO SIMPLE METHODS FOR THE RECOVERY OF SALMONELLA FROM FOOD FOR DETECTION BY PCR

W. Mark Barbour,\* Development Microbiologist, Food Quality Management and H. Zanecovsky, DuPont Co., Experimental Station, Building 228, Wilmington, DE 19880-0228

Recent years have seen the development of numerous foodborne pathogen screening methods which employ the polymerase chain reaction (PCR). The sensitivity of these methods often allow more rapid detection than standard methods or other rapid methods. However, the sample preparation procedures for PCR are often tedious and time-consuming. Alternatively, more simple sample preparation methods are seldom applicable to a wide range of foods due to the presence of inhibitors of the PCR reaction. We have developed two simple, universal procedures for preparation of microbial samples from food for PCR analysis. Briefly, the first method employs the capture of target organisms on a porous plastic substrate during the standard enrichment step. The solid support is then removed and washed to eliminate food debris. Following lysis of the bacteria by heating in the PCR reaction buffer, the lysate can be added directly to the amplification reaction. The second method uses a prefilter to remove large food debris, then a membrane filter to capture target bacteria. The filter is then washed and prepared for PCR as above. The efficacy of the two methods was compared using a wide variety of food samples seeded with low levels of *Salmonella*. Results show that *Salmonella* was detected in greater than 90% of the foods tested with either method, comparable to the conventional methods. We find both methods to be simpler and more rapid than most other sample preparation procedures, and to be applicable to a broad range of foods.

## COMPARATIVE STUDY FOR DETECTION OF LISTERIA MONOCYTOGENES IN FOODS BY A COLORIMETRIC ONA METHOD AND CONVENTIONAL CULTURE METHODS

Gregory W. Durbin,\* Senior Research Associate Scientist, Karen M. Keough and Gwen N. Reynolds, GENE-TRAK Systems Corp., 31 New York Avenue, Framingham, MA 01701

The performance of a colorimetric DNA hybridization assay was compared to conventional USDA or FDA culture methods for detection of *Listeria monocytogenes* in foods. Dairy and processed meat products were

inoculated with low levels of *Listeria innocua* and *L. monocytogenes* and enriched according to described methodology. Naturally contaminated meat, poultry and seafood samples were also tested. The DNA hybridization method was highly specific and detected 98% of the 223 samples positive for *L. monocytogenes*. The combined culture methods detected only 49% of the total positive samples. While demonstrating greater specificity, the DNA hybridization assay also offered a significant advantage in time to result over the culture methods. This study demonstrated that the DNA hybridization assay provides a rapid alternative to conventional culture methods for the specific detection of *L. monocytogenes* in foods.

#### RAPID ASSAY SYSTEM FOR THE DETECTION OF BETA-LACTAM RESIDUES IN MILK

Susan Faust,\* Research Associate, Scott Clark and Larry Chaney, IDEXX Laboratories, 1 IDEXX Drive, Westbrook, ME 04092

The SNAP™ Beta-lactam assay system detects residues in milk at or below the FDA tolerance levels for penicillin-G (5 ppb), cephalixin (20 ppb), amoxicillin (10 ppb), ceftiofur (50 ppb) and ampicillin (10 ppb). Randomized, coded samples of negative milk and milk spiked with tolerance levels of antibiotic (n = 30 replicates) were assayed on SNAP™ devices, scored visually and read with the ImageReader, a photodiode array reflectance detector. Assay turn-around time is 9 min, assays may be batched and little hands-on time is required. No false-positives (0/30) or false-negatives (0/30 for each antibiotic) were reported for either the reader or visual interpretation, thereby establishing sensitivity at tolerance levels at least 90% of the time with 95% confidence. This assay system meets the FDA certification requirements for beta-lactam residue detection in unprocessed milk.

#### REDUCTION OF HYDROXYMETHYLFURFURAL OF HONEY EXPOSED TO DIFFERENT SOURCES OF RADIATION

J. A. F. Faria,\* Professor, Ph.D., Campinas State University, P. O. Box 6121, Campinas, SP, Brazil

Official standard and international regulations have established maximum limits of HMF as one of the parameters for quality control of honey. The effects of light on the HMF content of honey was investigated by exposing honey sample and packages to different light sources. Transparent and translucent packaging materials were used. The light sources were fluorescent tubes arranged so as to simulate the spectral energy distribution similar to that of ordinary room illuminations, ultraviolet tubes and direct sunlight. The spectral characteristics of the packaging materials and of the honey used in the experiment were also determined. The photosensitivity of HMF was observed as a function of the light sources and barrier properties of the packaging materials. Light sources emitting the highest radiant energy (UV tubes) reduced the initial concentration of HMF to almost zero after a few hours of exposure. The relative effect of the packaging materials correlated well with their light transmission curves, the amber materials being the most effective in maintaining the initial HMF value. For the transparent materials, the reduction of HMF correlated directly with the light energy absorbed, sunlight being the most efficient.

#### ESTIMATION OF COLIFORM COUNTS USING THE BACT/ALERT® MICROBIAL DETECTION SYSTEM

Scott R. Jeffrey,\* Research Scientist I, Karen Read and Barbara Robison, Organon Teknika Corporation, 100 Akzo Avenue, Durham, NC 27712

Enumeration of coliforms is one method used to determine the quality of food products. Standard methods are laborious and require a minimum of 48 h for results. The Bact/Alert automated microbial detection system detects the presence of microorganisms by the colorimetric monitoring of CO<sub>2</sub> production. Each Bact/Alert culture bottle contains a colorimetric sensor which is monitored every 10 min by the Bact/Alert instrument. A growth detection algorithm determines the presence of microorganisms based on a sustained acceleration in CO<sub>2</sub> production. The following study was undertaken to determine the suitability of using the Bact/Alert system for the estimation of coliforms in food samples.

Ground beef and milk were obtained locally from retail outlets. In order to establish a wide range of bacterial levels, samples were artificially contaminated with mixtures of *Escherichia coli*, *Citrobacter* sp. and *Klebsiella pneumoniae*. A correlation between the log<sub>10</sub> MPN and the time until

the instrument called the sample positive (time to detection, TTD) was calculated. For ground beef, the correlation was 0.93, for milk the correlation was 0.97. Samples containing 10 coliforms/g were detected in less than 30 h using the Bact/Alert. These results indicate that the Bact/Alert system may be used to rapidly estimate the number of coliforms in a food sample.

#### USE OF THE BACT/ALERT® MICROBIAL DETECTION SYSTEM TO MONITOR STERILITY OF ASEPTICALLY PROCESSED PUDDING

Barbara J. Robison, Section Head, Organon Teknika Corp., 100 Akzo Avenue, Durham, NC 27712

The Bact/Alert is a microbial detection system consisting of a fully automated instrument and a unique culture bottle containing a built-in colorimetric sensor. As organisms grow and produce CO<sub>2</sub> in the medium, the sensor changes from blue-green to yellow. A semi-permeable membrane separates the sensor from the medium and allows diffusion of CO<sub>2</sub> only. The instrument continuously monitors changes in the sensor by reflectance and flags positives via a computer system. The system has been in use since 1990 to detect the presence of organisms in blood.

The purpose of this study was to determine the efficiency of the Bact/Alert in detecting organisms artificially inoculated into commercial aseptic pudding products. The inoculum consisted of a cocktail of three organisms previously isolated from spoiled pudding. Levels of inoculum were determined by plate count. Uninoculated controls were also tested. Inoculated product was not pre-incubated prior to testing in the Bact/Alert. All seven flavors of pudding inoculated with 6 cells/4 ounce cup were detected by the Bact/Alert in <36 h. Higher levels of inocula were detected more quickly. The results indicate that the Bact/Alert system can adequately monitor sterility of these aseptically processed products.

#### EFFICACY OF THE MICROCOLONY IMMUNOBLOT TECHNIQUE TO DETECT HEAT-INJURED LISTERIA MONOCYTOGENES

J. R. Patel,\* Graduate student and L. R. Beuchat, Center for Food Safety and Quality Enhancement, University of Georgia, Griffin, GA 30223

Only a few techniques have addressed the recovery of heat-injured *Listeria monocytogenes* from foods. We evaluated the microcolony immunoblot technique (MIT) using *L. monocytogenes*-specific monoclonal antibodies for its suitability to enumerate heat-injured cells. Performance was also compared with conventional plating. Uninjured and heat-injured *L. monocytogenes* Scott A cells were inoculated into Fraser broth (FB) supplemented with 400 µg of catalase ml<sup>-1</sup> or 0.01 unit of Oxyrase® ml<sup>-1</sup> and incubated at 30°C for 6 h. Populations of viable cells were determined by MIT and by surface plating on tryptose phosphate agar (TPA). Essentially all unheated (control) cells were detected within 24 h using the immunoblot technique; 48 h were required to easily detect colonies on TPA. Recovery was enhanced when heat-injured cells were cultured in FB supplemented with catalase (90%) or Oxyrase® (88%) compared to the control (74%). Results indicate that MIT in conjunction with enrichment in presence of oxygen scavengers enhance the detection of heat-injured *L. monocytogenes*.

#### EFFECT OF TEMPERATURE AND CELL CONCENTRATION ON RADIOSENSITIVITY OF LISTERIA MONOCYTOGENES

Linda S. Andrews,\* Research Associate, D. L. Marshall and R. M. Grodner, Department of Food Science, Louisiana Agricultural Experiment Station, Louisiana State University, Baton Rouge, LA 70803

Among foodborne pathogens, *Listeria monocytogenes* is more radiation resistant than gram-negative bacteria of the genera *Salmonella* and *Vibrio*. The purpose of this study was to determine if initial cell concentration and/or temperature at time of irradiation influences the radiosensitivity of *L. monocytogenes*. Concentrations of 10<sup>6</sup> and 10<sup>9</sup> CFU/ml of *L. monocytogenes* Scott A, were suspended in tryptic soy broth and exposed to 0-5 kGy gamma (1.25 MeV) radiation at 20, 4 and -80°C. Surviving cells under the various conditions were enumerated and irradiation D-values were calculated from linear regression curves. The irradiation D-value of 0.43 kGy for frozen (-80°C) cultures of 10<sup>6</sup> CFU/ml was significantly lower (p<0.05) than the D-values (0.58 and 0.62 kGy) for the same cell concentration at the other two temperatures (20° and 4°C, respectively). For higher cell concentration of 10<sup>9</sup> CFU/ml, a D-value of 0.42 kGy was obtained for both 4°C and -80°C that was significantly lower (p<0.05) than 0.50 kGy for 20°C suspensions.

With both cell concentrations, the lowest D-values were obtained when cells were irradiated in the frozen state. Thus temperature of irradiation and cell concentration influenced the radiosensitivity of *L. monocytogenes*.

#### THE DEVELOPMENT OF A PCR BASED ASSAY FOR THE DETECTION OF *SALMONELLA*

George Tice,\* Sr., Development Biochemist, Food Quality Management, M. A. Jensen, R. Jackson and J. C. Noszek, DuPont Co., Exper. Station, Building 228, Wilmington, DE 19880-0228

There is a need in the food industry for a rapid, highly sensitive and selective assay for the detection of *Salmonella* in both raw materials and finished products. A PCR based assay has been developed that is rapid (<3 h), sensitive ( $10^4$  CFU/ml) and selective (>99.7%).

*Salmonella* specific PCR conditions were optimized for concentrations of deoxynucleotides, primers, KCl, Mg<sup>2+</sup> and Taq polymerase. The selection of the *Salmonella* specific primers was based upon the ability to prime and amplify a highly conserved region of a genus specific DNA fragment. Final reaction optimization conditions were evaluated based upon the ability to detect 21 strains of highly polymorphic *Salmonella* strains. Polymorphic strains were defined as those strains of *Salmonella* which showed any polymorphic variation in the size and/or intensity of the amplification product. Using the current reaction conditions 99.7% of the 1400 *Salmonella* strains in our collection were detected. Over 100 non-*Salmonella* gram-negative enteric bacterial strains have been tested with the current assay conditions and <1% have yielded an amplification product.

With further development, this assay may provide the food industry with a rapid and sensitive method for the detection of *Salmonella*.

#### IDENTIFYING AND TYPING *LISTERIA* SPECIES WITH PATTERNS OF ECO R1 FRAGMENTS CONTAINING RIBOSOMAL RNA OPERON SEQUENCES

John A. Webster,\* Technology Consultant, Food Quality Management, E. M. Cole, J. L. Bruce, C. Iem and R. J. Hubner, DuPont Co., Experiment Station, Building 402, Wilmington, DE 19880-0402

A general method to characterize bacteria using genomic Eco R1 fragments labeled through hybridization with a ribosomal RNA operon from *Escherichia coli* was used to identify and type over 1,800 strains of *Listeria* species. Strains of *Listeria monocytogenes* and other species were studied with a system that includes molecular weight markers, a chemiluminescent probe label, electronic imaging, algorithms for mobility adjustments and a database. The patterns of labeled fragments were sorted according to similarity using correlation values. Polymorphic changes in the patterns permitted the formation of infrasubspecific clusters and these clusters were compared to the classification by serotyping. As a preliminary result, although strains of *L. monocytogenes* with serotypes reportedly linked to human disease 1/2a, 1/2b and 4 b are present in a majority of pattern types, 4b is predominantly represented in 3 pattern types. This system and classification of pattern types provides an additional tool to study pathogenic *Listeria* species.

#### A 43-HOUR TEST FOR DETECTING *LISTERIA* IN FOODS USING THE UNIPATH *LISTERIA* CLEARVIEW IMMUNOASSAY

R. Holbrook,\* T. A. Briggs, J. A. Anderson, J. A. Blades and P. Sheard, Unilever Research, Colworth House, Sharnbrook, Bedfordshire, UK

The Unipath *Listeria* Clearview Immunoassay (S. Parry, et al. this meeting) offers a rapid, sensitive, easy to use test for the detection of *Listeria* species in culture. Its reliable use for the examination of food and environmental samples requires the optimization of the culture enrichment system. Four *Listeria* primary enrichment broths - UVM1, Fraser Broth and two modifications of Fraser broth were evaluated for the recovery of damaged *Listeria monocytogenes*; and in combination with UVM2 and Buffered *Listeria* Enrichment broth (BLEB) for the detection of *Listeria* species in naturally contaminated foods. The most productive combination from 660 tests was evaluated in a trail in 10 Unilever laboratories. Results from over 1,000 tests gave >99% correlation between streaking BLEB onto Oxford agar or using the *Listeria* Clearview assay.

A user friendly, flexible 43-h presence/absence screening test comprises primary enrichment of sample in Modified Fraser broth (having half the concentration of selective agents) subculture to BLEB, both incubated at

30°C for 21 h; after which a 2 ml portion of BLEB is heated at 80°C for 20 min. (to release the B flagella antigen), and 120 µL of the cooled sample added to the Clearview device. It is read by eye 20 min later. Positive results need confirmation on Oxford agar.

#### THE RAPID CLEARVIEW™ *LISTERIA* IMMUNOASSAY FOR DETECTION OF *LISTERIA* SPECIES

Stephen H. Parry,\* T. Briggs, J. A. Blades, M. Gani and J. Piron, Unilever Research, Immunology Department, Colworth Laboratory, Sharnbrook, Bedfordshire, U.K.

This poster describes a novel rapid and simple immunoassay which detects *Listeria* species present in liquid culture media after selective enrichment. The format consists of a line of antibodies localized on a membrane, which, colored particles coated with second antibody are flowed past. If antigen is present the particles localize on the membrane in a sandwich reaction forming a blue line.

Monoclonal antibodies were raised against the common 'B' flagellar antigen of *Listeria*. After preliminary screening, ten antibodies were evaluated in the Clearview immunoassay format for specificity and sensitivity. A pair of monoclonals were selected which exhibited excellent specificity against the main *Listeria* species with no demonstrable activity against non-*Listeria* species.

Antigen production and release were investigated and culture at 30°C gave optimal growth and flagella production, while heating food cultures at 80°C for 15 min released antigen and rendered the sample safe. Assays were performed simply by adding five drops of culture to the device which was allowed to develop for 15 min. A positive result, indicated by a blue line in the test window, was read visually and gave a test sensitivity of <10<sup>5</sup> organisms per ml.

The Clearview™ test that was carried out following two 21-h cultures of food samples shows excellent correlation with traditional culture methods with a result obtainable within 43 h (see accompanying poster by Holbrook et al). It is concluded that it offers significant practical advantages for rapid screening of food or environmental samples.

#### OPTIMIZATION OF COMMERCIAL STERILITY TESTING

Mary Robart,\* Senior Microbiologist, Jarus David, Sue Alles, Tammy Weaver, Shen-Youn Chang and Terry VanArman, Gerber Products Company, 445 State Street, Fremont, MI 49413

Samples of USDA thermally processed canned foods must be incubated at 35°C for 10 days. Extended hold of production results in economic loss due to warehousing costs and inflexibility in scheduling of production and can slow introduction of new products. Underprocessed variables of a puréed baby dinner were prepared to develop methods for thermal process validation. Samples were tested for commercial sterility immediately following the thermal process and after incubation at 35°C for 1 to 5 days and at 10 days by the following methods: 1) AOAC method (subculture in PE2 broth and Tryptone broth); 2) Incubation of a Tryptone broth subculture at 35°C followed by bacterial ATP analysis (Lumac); and 3) Incubation of a Wilkins-Chalgren Anaerobe broth culture at 35°C followed by conductance assay (Bactometer). Results indicate commercial sterility testing is useful for evaluating thermal process delivery. Recovery can be improved by 1 to 4 days of preincubation of the jar, followed by aerobic subculture in an enriched broth with instrumented detection of bacterial growth.

#### COLD TEMPERATURE STRESS RESPONSE OF PSYCHROTROPIC *BACILLUS CEREUS*

Elaine D. Berry,\* Graduate Student and Peggy M. Foegeding, North Carolina State University, Department of Food Science, Box 7624, Raleigh, NC 27695-7624

The properties of both heat-resistance and ability to grow at refrigeration temperatures combine to make psychrotropic *Bacillus cereus* an organism of significance to the food industry, from both a shelf-life and a public health standpoint. In order to study how *B. cereus* regulates cold temperature growth, cells were pulse-labeled for 1 h with 100 µCi <sup>35</sup>S-methionine (ca. 70%) and <sup>35</sup>S-cysteine (ca. 15%) during logarithmic growth at 30°C and 10°C, and following a shift from 30°C to 10°C. Proteins in sonicated whole cells extracts were separated by SDS-PAGE, and labeled proteins



were visualized by autoradiography. Protein band patterns indicated that *B. cereus* exhibits a cold stress response via changes in protein synthesis when shifted from 30°C to 10°C; notable is the increase in synthesis of a ca. 16.6 kDa protein.

#### MODEL FOR THE NON-THERMAL INACTIVATION OF *LISTERIA MONOCYTOGENES* IN A REDUCED OXYGEN ENVIRONMENT

Robert L. Buchanan\* and Marsha H. Golden, U.S. Department of Agriculture, ARS, Eastern Regional Research Center, 600 E. Mermaid Lane, Philadelphia, PA 19118

The non-thermal inactivation of *Listeria monocytogenes* under anaerobic conditions was studied to develop models to complement one developed for aerobic conditions. Brain Heart Infusion (50 ml) adjusted to NaCl (0.5-19.0%), lactic acid (0-2%) and NaNO<sub>2</sub> (0-200 µg/ml) was dispensed in 250-ml trypticizing flasks. The flasks were inoculated with three strains of *L. monocytogenes* to 10<sup>8</sup> CFU/ml. After N<sub>2</sub> flushing, the flasks were sealed and incubated (5° - 42°C) for 90 to 120 days. Samples were removed via the sampling septum, viable counts performed, and inactivation curves generated using linear and non-linear primary models. A total of 197 curves were generated, representing 155 variable combinations. The data were used to develop quadratic and cubic models that correlated the logarithm of the "time to a 4-D inactivation" with temperature × NaCl × NaNO<sub>2</sub> × lactic acid and temperature × NaCl × NaNO<sub>2</sub> × pH. While there was a relatively high degree of variability, the models provide a means for estimating the pathogen response to acidic environments. Comparison of the aerobic and anaerobic models indicated that *L. monocytogenes* behaved similarly under the two conditions.

#### THE SYNERGISTIC EFFECT OF SODIUM ACETATE OR SODIUM PROPIONATE USED IN COMBINATION WITH EDTA AND ASCORBIC ACID ON THE INACTIVATION OF *LISTERIA MONOCYTOGENES*

Marsha H. Golden,\* Microbiologist, Robert L. Buchanan and Richard C. Whiting, U.S. Department of Agriculture, ARS, ERRC, 600 East Mermaid Lane, Philadelphia, PA 19118

Several organic acids approved for use as food additives inhibit *Listeria monocytogenes*. Although there has been research on the effects of individual compounds, little information exists on their efficacy when used in combination with other additives. As a result of strong responses in preliminary studies, we undertook to characterize the effects of combinations of 90% sodium acetate or sodium propionate with 8% EDTA and 2% ascorbic acid on the inactivation of a three strain mixture of *L. monocytogenes*. Activity was assessed in Brain Heart Infusion at various concentrations (0-2%), pH values (3.0-4.5), and temperatures (4-28°C). Samples were removed for up to 150 days, and viable counts determined. Survivor curves were fitted using a non-linear inactivation model, and used to calculate D-values, lag periods and "times to a 4-D (99.99%) inactivation" (T<sub>4D</sub>). Inactivation rates were directly related to concentration and temperature, and inversely related to pH. The fastest T<sub>4D</sub> was 16.4 h with 2% propionate/EDTA/ascorbate mixture at pH 3.0 and 28°C. The equivalent HCl-adjusted control had a T<sub>4D</sub> of 120.2 h. These data suggest that the microbiological safety and stability of foods could be enhanced by the identification of food additives that act synergistically to control foodborne bacteria.

#### *AEROMONAS HYDROPHILA* AND PSYCHROTROPH POPULATION OF CAGE- AND POND-RAISED CHANNEL CATFISH

Yao-wen Huang,\* Assistant Professor, Chung-yi Huang and Gary Burtle, Department of Food Science and Technology, University of Georgia, Athens, GA 30602-7610

Channel catfish (*Ictalurus punctatus*) grown in cage and pond culture were collected from the same pond. Psychrotrophic bacteria and *Aeromonas hydrophila* from both pond water and fish were assessed. Microbial changes on fillets held in overwrapping and vacuum skin packaging were determined during 16 days of storage at 4°C. Protein, lipid, moisture and ash contents of cage-raised catfish were 17.48, 3.68, 79.07 and 1.02%, respectively, while those of pond-raised catfish were 17.38, 3.37, 79.47 and 1.01, respectively. Initial skin surface and viscera of pond-raised catfish had significantly higher (p<0.05) levels of both psychrotrophic bacteria and *A. hydrophila* than that of cage-raised catfish. On both cage- and pond-raised catfish, bacterial counts

were reduced by filleting and washing. Cage-raised catfish fillets held in both overwrapping and vacuum skin packaging had significantly lower levels of *A. hydrophila* and psychrotrophic bacteria than did pond-raised catfish fillets after 4 days of storage; however, no significant difference was found on the 16th day, regardless of raising and packaging method.

#### THE USE OF RESPONSE SURFACE METHODOLOGY TO MODEL NON-LINEAR SURVIVAL CURVES AND TO PREDICT THE EFFECTS OF TEMPERATURE, PH AND SODIUM CHLORIDE ON THE HEAT RESISTANCE OF *LISTERIA MONOCYTOGENES* SCOTT A

Richard Linton,\* W. H. Carter, C. Gennings and M. D. Pierson, Department of Food Science, Virginia Tech University, Blacksburg, VA 24061-0418.

Numerous examples of non-linear survival curves for bacteria exposed to heat have been reported. In this study, a modified form of the Gompertz equation was used to mathematically model non-linear survival curves for *Listeria monocytogenes*. Cells were heated at 50, 55 or 60°C in 0.1 M KH<sub>2</sub>PO<sub>4</sub> buffer supplemented with 0, 2 or 4% NaCl and adjusted to pH 5, 6 or 7. Using the Gompertz equation and response surface methodology, single and multiple interactions, which influenced the heat resistance of *L. monocytogenes* were determined. Significant (p<0.05) factors which contributed to an increase in heat resistance included increased pH and increased NaCl concentration. A decrease (p<0.05) in heat resistance was attributed to increased temperature and increased time. Significant (p<0.05) factor interactions which affected the heat resistance included: (NaCl × time), (temperature × NaCl × time) and (temperature × time). Mathematical modeling can be used to more accurately estimate heat resistance rates and to determine important factors which affect the heat resistance of bacteria.

#### VALIDATION OF PREDICTIVE MATHEMATICAL MODELS TO DEMONSTRATE APPLICABILITY TO FOODS

Isabel Walls,\* Project Microbiologist II, V. N. Scott and D. T. Bernard, National Food Processors Assn., 1401 New York Avenue, NW, Washington, DC 20005

In predictive microbiology, mathematical models are used to predict the growth of microorganisms under different conditions. Before use, models must be validated, to demonstrate their applicability to foods. Growth studies were performed in commercially available sterile homogeneous foods using *Listeria monocytogenes* at 20°C and 35°C with 0%, 3% and 4.5% added NaCl, *Listeria innocua* at 20°C and 35°C with 1%, 3% and 4.5% added NaCl and *Staphylococcus aureus* at 12°C, with 1% and 5% added NaCl and at 20°C and 35°C with 1%, 10% and 15% added NaCl. Growth data were fitted to the Gompertz equation and resulting growth kinetics were compared with predictions from models developed at the USDA-ARS. Lag phases were shorter than predicted for *L. monocytogenes* by 10% to 59%, for *L. innocua* by 50% to 80% and for *S. aureus* by 23% to 70%. Growth rates for *L. monocytogenes* ranged from 50% less to 24% greater, for *L. innocua* from 16% less to 40% greater and for *S. aureus* from 28% to 58% greater. Therefore, for the sterile foods used, under the growth conditions used, the models gave predictions that were less conservative than necessary to assure the safety of food. Further research is needed to determine whether this is true for non-sterile foods.

#### THE ECONOMICS OF FEDERAL HACCP REGULATIONS

David J. Zorn,\* Ph.D., Economist, Food and Drug Administration (HFS-726), 200 C Street, S.W., Washington, DC 20204

This paper will describe and summarize the survey and other research that FDA has conducted on the costs to industry of implementing HACCP-type regulations (\$23,900 per manufacturing plant in the first year and \$15,000 in the following years totaling \$139 million dollars in the first year) and the estimations of potential benefits in terms of illnesses prevented (5,000 - 19,000 annually, valued at \$15 - \$75 million), valued using willingness-to-pay plus medical cost methodology, and in terms of increased seafood consumption (673 deaths from heart disease and cancer prevented over 10 years, valued at \$3 billion). The agency's perspective on benefits to industry in export markets and to government by allowing for more effective enforcement will be presented. The results will describe the agency's concern for the financial consequences for small businesses in complying with HACCP regulations and the various options open to the agency for implementing HACCP regulations (e.g., regulatory phase-in by risk cohort).

## AN EXPERT SYSTEM FOR HACCP IMPLEMENTATION

Felix Barron,\* Ph.D. and James C. Acton, Clemson University, Department of Food Science, 224 Poole Agricultural Center, Clemson, SC 29634

Hazard Analysis Critical Control Point (HACCP) is based on well established guidelines for identification of hazards, critical control points (CCP's), their deviations and corrective actions. The objective of this study was to develop an expert system (computer model) to act as an effective recording system for HACCP evaluations in a commercial bakery. "VP-Expert®", "Word Perfect®", and "Flow Charting®" are software packages that were used in the development of the "HACCP-Expert System" (HACCP-ES). The "HACCP-ES" was developed using a personal computer (PC) to generate hardcopies of flow diagrams, worksheets and corresponding HACCP reports of a filing system. Completed reports can be available for auditing or inspection purposes. The "HACCP-ES" was designed to start with a warning statement to the user indicating CCP's to be monitored "today" and the status of incomplete CCP reports. It also guides the user in locating CCP's in a particular flow diagram, as well as identifying corrective actions needed to control CCP's. Final reports are generated to be kept on file. The "HACCP-ES" proved to be an effective management tool to set up HACCP programs and to create and maintain HACCP reports within the commercial bakery. The "HACCP-ES" has the flexibility to create other HACCP programs for specific food processing industries.

## INFLUENCE OF TEMPERATURE ON HEMORRHAGIC *ESCHERICHIA COLI*: VEROTOXIN PRODUCTION AND MINIMUM TEMPERATURE OF GROWTH

Samuel A. Palumbo,\* Microbiologist, Frankie J. Schultz and Aaron C. Williams, U.S. Department of Agriculture, Eastern Regional Research Center, 600 E. Mermaid Lane, Philadelphia, PA 19118

Hemorrhagic *Escherichia coli* has emerged as a major foodborne human pathogen. In general, its culture characteristics are similar to non-pathogenic strains. Refrigeration of fresh foods, particularly red meats, represents one means of controlling the growth of pathogens in these foods. However, there are no data on the effect of temperature on the growth of hemorrhagic *E. coli* and on verotoxin production. Using BHI broth in a temperature gradient incubator set at 5 to 50°C, we determined time to visible turbidity for 15 O157:H7, O26:H11 and O111:NM strains. At this point, samples were removed for verotoxin assay. The minimum temperature of growth ranged from 6.9 to 13°C, with 10 strains growing at 9.0-9.5°C. Except for the two O111:NM strains, verotoxin was produced at all growth temperatures. Production was a time-temperature relationship, with more verotoxin produced at higher temperatures. Holding foods at 5°C should prevent hazards from this organism.

## APPLICATIONS FOR PREDICTIVE MICROBIOLOGY SYMPOSIUM

### OVERVIEW — RISK ASSESSMENT AND PREDICTIVE MICROBIOLOGY

Robert L. Buchanan, Microbial Food Safety Research Unit, U.S. Department of Agriculture, ARS Eastern Regional Research Center, Philadelphia, PA 19118

Microbiological risk assessments require knowledge of two equally important areas. The first is information on the dose-response relations that link the incidence of host responses such as infection, morbidity, or mortality with the quantitative levels of a pathogen. The second half of a risk assessment is exposure data, identifying the levels of a pathogen to which a population is being challenged. To date, the microbiological risk assessments that have been performed have assumed that the level of the microorganism is unchanging. This assumption is generally inappropriate for food systems, and this has limited the usefulness of this approach. However, the increased availability of predictive microbiology models provides a means for quantitatively assessing the impact of food systems on pathogen levels, thereby allowing more sophisticated exposure estimates. By combining these two approaches, it should be possible to begin mathematically assessing the public health impact of modifying food processing operations or requirements.

## APPLICATIONS FOR PREDICTIVE MICROBIOLOGY

Thomas A. McMeekin,\* Professor, and T. Ross, Department of Agricultural Science, University of Tasmania, Hobart, Tasmania, 7001, Australia

In the last decade the concept of predictive microbiology has developed rapidly through the initial phases of experimental design and model development and the subsequent phase of model validation. For some microorganism/food combinations sufficient confidence, now exists to indicate substantial benefits to the food industry from the use of predictive models.

Several types of devices are available to monitor and record environmental conditions (particularly temperatures). These 'environmental histories' can, using predictive models, be interpreted in terms of microbial proliferation. The current challenge is to provide delivery systems which combine ease-of-use, reliability and security, providing the industrial user with the ability to make informed and precise decisions regarding the quality and safety of foods. This type of technology will be demonstrated using "PSEUDOMONAS PREDICTOR," a spreadsheet-based application developed at the University of Tasmania.

### FOOD MICROMODEL UPDATE

Terry A. Roberts, Head, Microbiology Department, Institute of Food Research, Reading Laboratory, Early Gate, Reading RG6 2EF, UK

Over the past 5 years the Ministry of Agriculture Fisheries and Food has funded a coordinated research program into modeling the growth and survival responses of foodborne bacterial pathogens. Models are developed in laboratory media, then "validated" against data gathered from the literature or, where insufficient data are available, against systematic inoculated food studies. Models accessible on a main-frame computer proved unpopular with the food industry and a PC-based version will be marketed in mid-1994. Over the same period, a European program under FLAIR has led to widespread uptake of modelling as a way forward in ensuring food safety and understanding, and perhaps controlling food spoilage.

### MODEL VALIDATION (AND CONFIDENCE IN MODELS) — AN INDUSTRY PERSPECTIVE

Martin B. Cole,\* P. J. McClure, P. J. Stephens and K. W. Davies, Unilever Research, Sharnbrook, Bedfordshire, U.K.

Techniques for the development of mathematical models in the area of predictive microbiology have improved, allowing better and more accurate descriptions of microbial responses to particular environmental conditions to be made, thus improving confidence in the resulting predictions. Using automated data capture techniques and methods for studying the responses of single cells, it is possible to assess the importance of biovariability to the way we interpret microbial data for modelling and risk assessment purposes.

A number of factors associated with the design of experiments for modelling make the resulting model predictions 'fail safe.' In other words, where the model is inaccurate it will predict faster growth than would be expected in real foods. Factors that contribute to this include the choice of media, acidulant, strain and size of inoculum. Important stages to the acceptance of a model include mathematical testing for goodness of fit, tests for bias and validation with real food studies. Validation with literature data can be useful but often reveals marked deficiencies in the literature itself with many authors giving incomplete information about their foods, experimental designs and/or methods, and rarely generating data suitable for modelling. In most cases, models generated in laboratory media give predictions that are relevant to most food groups and show excellent agreement with data from studies in real foods.

### COLD STORAGE TEMPERATURE FLUCTUATIONS AND PREDICTING MICROBIAL GROWTH

Colin O. Gill, Agri-Food Canada Research Station, Bag Service 5000, Lacombe, Alberta, Canada TOC 1S0

The proliferation of bacteria indicative for health risks or growth of spoilage flora can be calculated by integrating product temperature histories with respect to models relating rates of bacterial growth to temperature. When temperature function integration is used to characterize the hygienic consequences of the control over temperature exerted during a process, temperature histories must be collected from the microbiologically contami-

nated regions of product that can be expected to experience the highest temperature for the longest periods. The models used for integration must be for appropriate organisms growing in the most favorable environment likely to exist within the product. Then, a process can be effectively characterized by the distributions of proliferation values calculated from the temperature histories obtained from >20 product units that passed through the process and were selected at random.

The application of the temperature function integration technique in assessments of commercial processes for the cooling, storage, transportation and display of meats, and the use of the technique in Hazard Analysis: Critical Control Point (HACCP) systems, are discussed.

#### **PREDICTIVE MICROBIOLOGY AND HACCP**

Philip H. Elliott, Campbell Soup Company, Campbell Place, Camden, NJ 08103

While both predictive microbiology and HACCP are still in their developmental stages as food safety tools, predictive models are available that are potentially useful in the development and maintenance of HACCP systems. When conducting a HACCP study, models can be used to assess the risk (probability) and determine the consequence of a microbiological hazard in food. The risk of a hazard is reduced and controlled within the HACCP framework by assigning Critical Control Points (CCPs) to the food process. By using predictive models, ranges and combinations of process parameters can be established as critical limits for CCPs. This has the advantage of providing more processing options while maintaining a degree of safety equivalent to that of a single set of critical limits. Validation testing of individual CCPs can be reduced if the CCP models were developed with a similar food type. Microbiological as well as mechanical and human reliability models, may be used to establish sets of rules for rule-based expert computer systems in the effort to automate the development of HACCP plans and evaluate the status of process deviations. Models can also be used in combination with sensors and microprocessors for real-time process control. If HACCP is a risk reduction tool, then predictive microbiological models are tools used to aid in the decision making processes of risk assessment and in describing process parameters necessary to achieve an acceptable level of risk.

### **REDUCTION OF FOODBORNE PATHOGENS ON POULTRY**

#### **SALMONELLAE IMPORTANCE AND DETECTION IN POULTRY FEEDS**

Amy L. Waldroup, Department of Poultry Science, University of Arkansas, Fayetteville, AR 72701

Numerous surveys conducted in the U. S., Canada and in European countries have demonstrated that a high percentage of feed ingredients and finished animal feeds, especially those containing animal by-products, are contaminated with salmonellae. The relationship of salmonellae in feed and salmonellae contamination of the processed poultry carcass has been suggested in several studies, but is still questioned in regards to importance to overall human health. However, in September 1990, CVM announced a goal of "zero tolerance" for salmonellae in feed ingredients and finished animal feeds. In order to monitor such a program, a rapid, sensitive and accurate analytical method for detection must be developed and utilized. Researchers are currently working to evaluate several commercially available "rapid" test kits and a modified-BAM cultural procedure. These procedures are being evaluated using an increased sample size and improved pre-enrichment, enrichment and selective plating media. An interlaboratory study will be conducted during the final phase of this study and will involve evaluation of artificially and naturally contaminated feed ingredients and finished feeds.

#### **CONTROL OF SALMONELLAE DURING POULTRY PRODUCTION**

J. S. Bailey, U.S. Department of Agriculture, ARS, Russell Research Center, Athens, GA 30613

Control of salmonellae colonization of broiler chickens during grow out is complicated because of the numerous sources of salmonellae, the lack of microflora in the gut of young chicks, and the conditions under which the chickens are reared. Serological identification of isolates from the different sources indicate that the hatching cabinet is possibly the most critical control point in preventing intestinal salmonellae colonization of chickens.

Microaerosoling of various chemicals into the hatching cabinet during hatch has proven effective in reducing the spread of salmonellae between chicks. Mucosal competitive exclusion cultures have effectively been tested in commercial integrated poultry companies in Puerto Rico and Georgia. In these studies, the incidence rate of salmonellae has been significantly reduced both intestinally in birds leaving the farm and on processed carcasses leaving the processing plant.

#### **THE APPLICATION OF PROCESS MODIFICATIONS, CHEMICAL TREATMENTS AND BIOPEPTIDES TO INHIBIT FOODBORNE PATHOGENS ASSOCIATED WITH POULTRY PRODUCTS**

Brian W. Sheldon, Department of Food Science, Box 7624, North Carolina State University, Raleigh, NC 27695-7624

An overview will be presented of past and ongoing studies to evaluate the effects of various process unit operation modifications, chemical treatments and inhibitory peptides on reducing the level of bacterial pathogens associated with fresh poultry products. Specific organisms that will be considered include *Salmonella*, *Campylobacter* and *Listeria* species. The impact of several unit operation modifications, including changes in scalding, eviscerator and chiller design on pathogen reductions will be addressed. Furthermore, summaries will be presented of both bench-top and commercial field trial studies that evaluated the inhibitory activity of various chemical treatments such as organic acid rinses, oxidizing agents (hydrogen peroxide, ozone, oxyhalogens), phosphates (trisodium phosphate), and quaternary ammonium compounds. The lethality of several inhibitor biopeptides against selected foodborne bacterial pathogens will be summarized. These include the bacteriocin, nisin, produced by the dairy fermentation organism *Lactococcus lactis* subsp. *lactis* and the amphibian-derived magainins. Advantages and disadvantages of these inhibitors will be considered relative to their efficacy, stability, safety, cost and impact on product quality.

#### **REDUCTION OF FOODBORNE PATHOGENS ON POULTRY BY TREATMENT WITH IONIZING RADIATION**

D. W. Thayer, Food Safety Research Unit, U.S. Department of Agriculture, ARS, NAA, FSR, 600 Mermaid Lane, Philadelphia, PA 19118

Foodborne pathogens such as *Salmonella*, *Campylobacter*, *Staphylococcus*, *Listeria* and *Escherichia coli* O157:H7 that may contaminate poultry can be greatly reduced in number and in some cases completely eliminated by treating the poultry with ionizing radiation doses less than 3.00 kGy. Survival of foodborne pathogens exposed to ionizing radiation is influenced by the radiation dose, and the temperature and the atmosphere inside the package during treatment. These factors have been investigated in sufficient detail that the survival of a given pathogen exposed to gamma radiation can be predicted. Results obtained with widely diverse products such as chicken legs and mechanically deboned chicken were remarkably similar. Poultry irradiated under proper conditions will be wholesome, may have extended shelf-life when properly refrigerated, and will have a significantly improved microbiological safety for the consumer.

#### **DEVELOPMENT OF A COMPREHENSIVE TOTAL QUALITY ASSURANCE PROGRAM FOR USE IN FULLY INTEGRATED POULTRY COMPANIES**

Michael C. Robach, Continental Grain Company, 3700 Crestwood Parkway, Duluth, GA 30136

In 1971, the principle of Hazard Analysis Critical Control Point (HACCP) was introduced at the National Conference on Food Protection. The HACCP system provides a more specific and critical approach to the control of biological, physical and chemical hazards than existing inspection and quality control procedures. The underlying principles of HACCP are the identification and assessments of hazards associated with growing, harvesting, processing, distribution and preparation of a food product; determination of critical control points to control or eliminate identifiable hazards; and the establishment of procedures to monitor critical control points. It is a specialized version of process control. An underlying principle of Total Quality Assurance program is the utilization of analytical tools such as statistical process control to identify and eliminate programs at the root cause. The TQA system has been developed and implemented covering process control points, quality control points and critical control points within an integrated broiler operation; a starting point in pursuit of a farm-to-table food safety assurance program.

## **FOODSERVICE INDUSTRY PERSPECTIVE ON PATHOGEN REDUCTION IN POULTRY**

Steven Grover, Assistant Director of Technical Services, National Restaurant Association, 1200 Seventeenth Street, N.W., Washington, DC 20036-3097

It is apparent that the foodservice industry has a critical interest in the safety, wholesomeness and public perception of poultry as well as all foods. Our industry has a great desire to provide and be provided with the safest poultry product possible. Generally, we support proposals which can demonstrably reduce the levels of foodborne pathogens in all foods.

Currently, our efforts in pathogen reduction are concentrated on providing the most up-to-date educational materials for foodservice operators and expanding the educational opportunities to as many operators as possible. We have created the "Industry Council on Food Safety" to further our pathogen reduction goals. Furthermore, we have worked to include Hazard Analysis Critical Control Point (HACCP) training in all our educational programs. Today, the expansion of our educational programs and inclusion of HACCP training is our best course for pathogen reduction.

## **PESTICIDES IN THE FOOD INDUSTRY SYMPOSIUM**

### **THE IMPACT OF SANITATION ON PEST CONTROL IN THE FOOD ESTABLISHMENTS**

Robert B. Gravani, Professor, Department of Food Science, Cornell University, 8-A Stocking Hall, Ithaca, NY 14853

Today, progressive food companies in all segments of the food industry are using innovative pest control programs based on the concept of integrated pest management (IPM). Integrated pest management is a unique, ecological approach to pest control that incorporates both prevention strategies and control measures.

One critical component of an effective IPM program in food operations is sanitation. Attention to the details of general sanitation throughout the establishment is essential, but special emphasis must be placed on the following areas: food receiving, facility structure and maintenance, food storage procedures; general housekeeping practices; garbage management; cleaning and sanitizing procedures; and employee knowledge of pests. The vital role that sanitation plays in the control of pests in food establishments will be highlighted in this presentation.

### **IPM—TRENDS IN PESTICIDE USE AND INDOOR ENVIRONMENTAL QUALITY**

Austin M. Frishman, AMF Pest Management Service, Inc., 30 Milken Road, Farmingdale, NY 11735

Less pesticide period is the trend in pest control, food not the exception—if anything—the focus of concern. Least toxic, Biodegradable, Biorationals and integrated pest management are all "buzz" words in vogue today. What does it really mean? Fewer pesticides but no increase in pests is the public demand. If the food industry can alter the menu of their recipes to create low cholesterol, low fat and low sodium, then they can develop alternatives methods of pest control. No longer shall the use of pesticides be the pillar of the foundation for pest control. It does not mean "no" pesticides, but it means precise application for precise needs when the alternatives are not available. As alternatives become available once again, the pesticides used will change.

With increased emphasis on environmental concern, the possible increased cost factor for less pesticide becomes more palatable. Vacuuming, heat, cold, mechanical barriers and even totally controlled indoor environments becomes more of a reality every day.

### **FUTURE OF PESTICIDES FOR USE IN FOODHANDLING ESTABLISHMENTS**

Jeffrey B. Tucker, B.C.E., Entomologist, Whitmore Research Laboratories, St. Louis, MO

While it is clear that there is a growing concern over the use of pesticides in general, food processors are being affected more greatly by subtle changes in product registration patterns and pesticide label language. Specifically, the instructions on pesticide labels regarding where and when they may be applied is becoming more precise and restrictive.

Some active ingredients are being restricted as to the type of location in which they can be used and inert ingredients are being more closely scrutinized with regard to their safe usage in food processing environments. One

of the major factors affecting efficacy of any insecticidal application in a food processing environment is the formulation utilized. In many cases, the formulation is more important with regard to product efficacy than is the active ingredient. New and more efficacious formulations are under development, however, it remains to be seen whether they will be permitted for use in and around food manufacturing facilities. Many of the active ingredients utilized in the future will require the user to adopt an entirely new paradigm concerning their expectations and for the role insecticides will play in insect management.

## **MEAT QUALITY AND SAFETY: CONCERNS AND SOLUTIONS THROUGHOUT DISTRIBUTION SYSTEMS SYMPOSIUM**

### **UPDATE ON EPIDEMIOLOGY OF FOODBORNE OUTBREAKS LINKED TO MEAT PRODUCTS**

Phyllis H. Sparling, D.V.M., M.S., Public Health Liaison, USDA, FSIS, CDC, Mailstop C-09, 1600 Clifton Rd., N.E., Atlanta, GA 30333

Foodborne disease outbreaks are discussed with an overview of the information each reporting system provides. A review of the available data on outbreaks linked to meat and poultry products is presented. Highlights of the epidemiology of *E. coli* O157:H7 and other foodborne pathogens are included.

### **MICROBIOLOGICAL CONTROLS FOR SAFETY AND QUALITY OF MEAT AND POULTRY PRODUCTS**

Dr. James L. Marsden, President, Meat Institute Foundation, 1700 N. Moore St., Ste. 1600, Arlington, VA 22209

Pathogenic organisms are often present in small numbers and are part of the natural microbial flora of live animals. Controlling the microbiological contamination of raw meat and poultry products is a primary area of focus in our industry. Research efforts are underway to identify control points in slaughter and processing operations which are designed to reduce the incidence of pathogens in raw products. New technology developed in part by the AMI Foundation research on organic acid carcass sprays and decontamination procedures offers practical means to reduce microbial contaminants. Research by the Foundation has also determined that low dose gamma irradiation is an effective technology for destroying pathogens in ground beef. Additional research is being conducted to determine the effectiveness of trisodium phosphate and high intensity pulsed light on destroying pathogens on beef carcasses. These technologies could be used at critical control points in a Hazard Analysis Critical Control Points (HACCP) process and the industry could verify these technologies through microbial testing. A pass/fail approach to microbial testing which is being supported by FSIS should not be used because it is not an effective means for monitoring critical control points and does not guarantee that products will be safer.

### **CONSUMER FOOD SAFETY EDUCATION**

Susan Conley, Director, USDA Meat and Poultry Hotline, 14th and Independence Ave. S.W., Room 2925, South Bldg., Washington, DC 20250

Today's consumer has been labeled "cooking illiterate" by the popular press. Industry and government research shows that people today know less about food preparation basics than ever before. Increasing incidence of foodborne illness has fostered a "farm-to-table" approach to foodborne pathogen reduction.

Each year the USDA Meat and Poultry Hotline receives some 130,000 calls from consumers who are concerned about food safety. Data from each inquiry is compiled and analyzed to assess consumer food safety knowledge and concerns. Consumer education programs are developed to address these concerns and to provide food safety education to the public. The Hotline and Consumer Education staffs of FSIS work in cooperation with academia, industry and media representatives to develop food safety programs.

## THE CHALLENGE OF HACCP IMPLEMENTATION IN FOODSERVICE OPERATIONS

Steven Grover, Assistant Director of Technical Services, National Restaurant Association, 1200 Seventeenth Street, NW, Washington, DC 20036-3097

Food Safety experts have for years (decades!) urged that Hazard Analysis Critical Control Point (HACCP) principles be applied to food protection programs in foodservice establishments. Recent public and media concern about food safety have rekindled interest in applying HACCP across the entire spectrum of food distribution, handling and service. Many foodservice organizations have adopted HACCP as the basis for their operational safety programs. A recent pilot program focused on seafood showed that HACCP could be effectively applied to a wide range of foodservice operations and that, overall, it produced beneficial results of reduced risk and improved quality.

However, the same pilot, along with anecdotal reports from operators, also indicate that the critical obstacles to HACCP implementation in foodservice settings are: excessive requirements for records; unfamiliarity of regulatory agencies and officials with daily operations of the restaurant, leading to unnecessary focus on non-critical components of the establishments and their processes; and the potential difficulty in broadening the HACCP program beyond a limited scope of processes or items.

As increased public and media pressure demands improvements in the food safety system, and especially in those protein foods of animal origin, it seems inevitable that HACCP applications must increase, at all points in the production and distribution chain. That increased use of HACCP must be carefully coordinated between and among all participants (producers; processors; distributors; retailers; foodservice; and regulatory agencies) in order to avoid simply exchanging one "check list" for another; and to avoid misleading the consuming public into believing that *Reduced* risk is "Zero Risk."

## SAFETY AND QUALITY OF MEAT PRODUCTS IN RETAIL OELI OPERATIONS

Dr. John Farquhar, Vice President, Science and Technical Services, Food Marketing Institute, 1750 K. Street N.W., #800, Washington, DC 20006

The primary focus of this presentation will be to review a critical control point model for ground beef. This program was developed for the Food Marketing Institute by ABC Research Corporation in Gainesville, FL. The review process for the model extended across a broad spectrum of affected allied industry groups. Recent concerns about *Escherichia coli* O157:H7 and other pathogens in our meat supply have created a need to control product quality from the farm to the consumer. The retail production and merchandising of ground beef depends on 1) receiving a safe raw material, 2) proper handling at the store level and 3) educating the consumer regarding proper storage and preparation.

In addition, there will be some discussions regarding recalling and trace back of questionable product in the public domain. This presentation will also review application of various HACCP models pertaining to meat products in delicatessens. This discussion will range from distribution and merchandising of these products (from formulation to display) as well as appropriate consumer advisories.

## GENERAL SESSION — THE NEW FDA FOOD CODE: HOW WILL WE IMPLEMENT IT?

### THE NEW FOA FOOD CODE

John E. Kvenburg, Division of Cooperative Programs, Center for Food Safety and Applied Nutrition, Food and Drug Administration, Washington, DC 20204

The new 1993 Food Code contains the Food and Drug Administration's latest advice on preventing foodborne disease in restaurants, food markets, institutions and food vending locations. Included are new recommendations for safeguarding public health and assuring that food is unadulterated and honestly presented when received by the consumer. New provisions address management knowledge, employee health, avoiding hand contact with ready-to-eat cooked food, modified temperatures for cooking and holding food, Hazard Analysis Critical Control Point (HACCP) based variances for food processing at retail and consumer information. The document also provides references and the public health reasons supporting the new standards. The model code is offered for adoption by local, state and federal governmental jurisdictions for administration by the various departments, agencies,

bureaus, divisions and other units within each jurisdiction that have compliance responsibilities for the retail segment of the food industry.

### THE RESTAURANT INDUSTRY PERSPECTIVE

Steven Grover, Assistant Director of Technical Services, National Restaurant Association, 1200 Seventeenth Street, NW, Washington, DC 20036-3097

The underlying rationale of the Food Code was to update and modernize existing model codes, reflecting the most current scientific information on food protection at the point of sale to customers, and simultaneously, to combine existing model codes covering foodservice, retail stores, and vending machine operations into a single document to reflect the changing markets of these three segments and to encourage inter-jurisdictional uniformity of regulation.

Food Code 1993 certainly combines the three segments, and, to at least some extent, has applied more modern scientific thought to its requirements.

Ideally, this should lead to improved uniformity and flexibility, as the code admirably recognizes alternative approaches and variances, and is more accommodating to Hazard Analysis Critical Control Point (HACCP) approaches.

However, a number of the regulatory discrepancies between the market segments remain, and the Food Code has included several new requirements which will be difficult and disruptive in daily application. Many questions remain to be answered about definitions, interpretations and scope of coverage. The final evaluation of Food Code 1993 will probably be determined by the degree to which the code is responsive to inquiry, interpretation and change; and in the degree to which it is adopted by regulatory agencies and the foodservice and retail industries as the normal operating standard.

### THE FOOD STORE PERSPECTIVE

Dr. John Farquhar, Vice President, Science and Technical Services, Food Marketing Institute, 1750 K. Street N.W., #800, Washington, DC 20006

The long awaited FDA recommendations for preventing foodborne disease were recently published as the 1993 Food Code. With 10,000 deaths and more than 24 million illnesses attributed to foodborne diseases each year, this code is intended to assist regulatory agencies, at all levels of government, in implementing food safety controls for retail food stores, foodservice establishments and vending operations.

Essentially, the Food Code has been updated and combined into a single document three former editions that separately covered foodservice establishments (such as restaurants), food vendors and food stores. The Food Code is not federal law or regulation, nor is it preemptive of State and/or local laws. Rather, the Code represents FDA's best advice for a uniform system of regulation to assure that food at the retail level is safe and properly protected.

State and local regulatory agencies will implement the code as follows: 1) enact into law by a State legislature; 2) promulgate as regulation (if a State legislative body has delegated rule-making authority); or 3) adopt as an ordinance, if a local legislative body has been delegated rulemaking authority or regulatory powers.

It is obvious that the FDA is making changes in its food safety strategy. The focus is now on preventing problems in store operation rather than detecting them in the finished product. Industry is being regulated to take more responsibility for preventing foodborne disease and the regulators must increase their effectiveness at monitoring industry's efforts.

The FDA is putting a strong emphasis on the Hazard Analysis Critical Control Point (HACCP) concept and terminology. HACCP is a system for ensuring food safety that involves identifying and monitoring the critical points in food preparation where the risks of foodborne hazards (microbial, chemical and physical) are greatest. The FDA is working to make HACCP the basis for its safety regulations.

This presentation will review FMI's new "Code Learning Module Series" which is currently being developed. Special emphasis will be placed on the need for education, foodhandler certification and HACCP orientation.

### THE VENDING MACHINE INDUSTRIES PERSPECTIVE

L. Eils, National Automatic Merchandising Association, 20 N. Wacker Dr., Ste. 3500, Chicago, IL

At first glance, the FDA 1993 Food Code looks like any other regulation dealing with the preparation and serving of food. However, as one begins to

## SCIENTIFIC POSTER SESSION

acquaint themselves with the details of the Code they soon find out there are several new requirements, which have been introduced which may require dramatic changes in the vending industry should the Food Code be implemented. These areas of concern to vending deal with management, employee health, personal cleanliness, temperature, preventing contamination and lastly, discarding ready-to-eat, potentially hazardous food in a vending machine. Before this Code can be implemented by the vending industry, these and several other items will need to be reviewed in depth as to how they will impact the industry regarding labor costs, additional training and equipment changes. We feel some changes in the Code will be needed before we can support full implementation of the document. A true cost-benefit relationship needs to be shown before implementation can take place.

### THE AGRICULTURAL AGENCIES PERSPECTIVE

E. C. Heffron, D.V.M., Director, Food Division, Michigan Department of Agriculture, 611 West Ottawa, P.O. Box 30017, Lansing, MI 48909

The Model Food Code is a model, an instrument to enhance raising regulatory and industry food safety standards. The code articulates prevention through the control of key susceptible processes. For the first time, a uniform set of model requirements, such as cooking and holding temperatures govern similar processes whether related to foodservice, vending devices or retail food establishments.

This code is a model based on contemporary prevention objectives, not comprised to potentially gain greater and more uniform jurisdictional acceptance, (not withstanding some jurisdictions) and may, for a myriad of reasons, desire to deviate in some manner from the model in its entirety. The successful food safety experience by industry and to a limited degree by regulators of the concepts detailed in the code are supplemented by provisions to assure accurate representations to consumers.

This model is flexible to allow jurisdictions necessary changes or additions and the most applicable enforcement remedy. Importantly, the Model Food Code is based on broad input, experience and advisement of the industry, food and public health regulators, and related legal sources and in a relatively easy to read format.

### THE HEALTH AGENCIES PERSPECTIVE

Daniel Sowards, Chief, Food Branch, Division of Food and Drugs, Texas Department of Health, 1100 W. 49th Street, Austin, TX 78756

The long awaited Food Code has been published. Since 1987, many individuals, including regulatory officials and industry representatives, have had an opportunity for input into the drafting of the document. Several FDA commissioned State officials, including myself, were permitted to preview and provide additional comments on the final draft in May of 1993. Consequently, few concerned have been without opportunity to provide comments.

The Food Code, in a manner of speaking, is a scientific masterpiece. The FDA appears to have done an excellent job of incorporating good science, including new technology such as vacuum-packaging and sous vide, employee health, bacteriology and toxins, into the Code. The problems with the Code are with the transference of this science into a code which can be enforced in practice.

HACCP is a great concept, and inspections based upon HACCP principles have been conducted by both State and local health officials for years. It is another matter altogether for the retail industry—especially the smaller independents—to develop their own HACCP plans. Also, without mandatory manager certification, how will we enforce the educational aspects of the Code? Can we adequately enforce double handwashing? Should we exclude "bed and breakfasts?" Do we have the authority to review HACCP plans and complaint files? Is "summary closure" legal? Where will the manpower come from to review "mini" HACCP's in order to issue variances? Will regulated industry be able to comply with the new refrigeration temperatures? What wording should be used for the undercooked/raw food "advisory," and how should the customer be advised? These and many other questions remain. If satisfactory responses are not forthcoming, will the Food Code receive wide acceptance? And if not, then the uniformity we currently have will no longer exist and in its place will be a hodgepodge of non-uniform does, stretch, from coast to coast and border to border.

### PURIFICATION AND CHARACTERIZATION OF A BACTERIOCIN PRODUCED BY *CARNOBACTERIUM PISCICOLA* LK5

Lori K. Bagi,\* Microbiologist, and Robert L. Buchanan, USDA, ARS, East. Reg. Res. Center, 600 East Mermaid Lane, Philadelphia, PA 19118

A bacteriocin produced by *Carnobacterium piscicola* LK5 was purified and characterized. The bacteriocin is proteinaceous and inhibitory to *Listeria monocytogenes*. LK5 bacteriocin was isolated by adsorption to the producer cell surface and selective release at low pH. The released bacteriocin was dialyzed using 3,000-molecular-weight-cutoff membrane and concentrated by freeze-drying. Freeze-dried preparations were analyzed by SDS-urea-PAGE overlaid with *L. monocytogenes*, and a single band of activity of approximately 4.0 kDa was present. Silver strained SDS-urea-PAGE gels showed three bands, but none corresponded to the activity brand. However, activity was eluted from gel slices of the 4.0 kDa region of an unstained gel. As an initial step in determining the location of the gene for bacteriocin production, *C. piscicola* LK5 DNA was isolated and purified. No plasmid DNA was evident suggesting that bacteriocin production is chromosomally mediated.

### BIOFILM FORMATION BY *ESCHERICHIA COLI* O157:H7 ON STAINLESS STEEL SURFACE: EFFECT OF CHEMICAL AGENTS

Ratih Dewanti, Research Assistant, Food Research Institute, Department of Food Microbiology & Toxicology, University of Wisconsin-Madison, Madison, WI 53706

Biofilm formation in food processing environments can serve as a source of contamination. We have found that *Escherichia coli* O157:H7 can form biofilms on a stainless steel surface. The effects of chemical agents on bacterial attachment and biofilm detachment were examined. Significant decrease ( $p < 0.025$ ) in attachment was observed in the presence of proteases, concanavalin A, Tween 20, deoxycholic acid and EGTA. Only Tween 20 promoted significant biofilm detachment. Sodium meta periodate, cetyl trimethyl ammonium bromide and benzalkonium chloride were found to inactivate biofilm bacteria without removing the adherent cells. Preliminary studies indicate a 43 KDa outer membrane protein (OMP) was present in biofilm bacteria, but absent in planktonic cells. Biofilm bacteria were more resistant to sanitizing agents as compared to their planktonic. This study suggested that properties of biofilm bacteria are different from planktonic cells and that OMP may be involved in attachment leading to biofilm foundation.

### COOLING RATE AND OUTGROWTH OF *CLOSTRIDIUM PERFRINGENS* SPORES IN COOKED GROUND BEEF

Vijay K. Juneja,\* Lead Scientist, Microbiologist, O. P. Snyder and Brian S. Eblen, USDA, ARS, ERRC, 600 East Mermaid Lane, Philadelphia, PA 19118

The ability of *Clostridium perfringens* spores to outgrow into vegetative cells was studied to determine a safe cooling rate for cooked beef. Beef samples were inoculated with a cocktail of three strains of heat-shocked *C. perfringens* spores (NCTC 8238, NCTC 8239 and ATCC 10288), vacuum-packaged and heated in a stirred water bath to 60°C in 1 h. Samples were cooled through the temperature range of 54.4°C to 7.2°C at rates varying from 6 to 18 h. Samples were removed at various times during cooling in order to determine if the spores had germinated and multiplied. The samples were plated on tryptose-sulfite-cycloserine agar and incubated anaerobically at 37°C for 48 h. No growth was observed up through 15 h cooling time. With an 18 h cooling time, *C. perfringens* spores germinated and grew from an inoculum of approximately  $1.5 \log_{10}$  to about  $6.0 \log_{10}$  CFU/g. These data indicate that pasteurized cooked beef must be cooled to 7.2°C in 15 h or less to prevent *C. perfringens* foodborne disease outbreaks.

### ISOLATION AND CHARACTERIZATION OF ENTEROCIN EL1 A BACTERIOCIN PRODUCED BY A STRAIN OF *ENTEROCOCCUS FAECIUM*

Wanda J. Lyon,\* Assistant Professor, Elsa A. Murano and Dennis G. Olson, Iowa State University, 2312 Food Sciences Building, Ames, IA 50011

A meat isolate, identified as *Enterococcus faecium* L1, was found to produce a bacteriocin designated enterocin EL1. Enterocin EL1 was found to be active against a narrow spectrum of microorganisms, inhibiting all tested strains of *Listeria*. Identification of the producer strain was determined phenotypically by biochemical and morphological tests. Enterocin EL1 was found to be heat-stable, sensitive to several proteolytic enzymes, and stable at pH 2-11. Adsorption of bacteriocins to producer cells appears to be dependent on the ionic interaction of the bacteriocin to the cell surface at various pHs. By changing the pH of the extraction buffer, enterocin EL1 was extracted from *E. faecium* L1 cells in a concentrated form. Enterocin EL1 isolated by cell extraction was resolved by sodium dodecyl-sulfate-polyacrylamide gel electrophoresis to be a protein with approximate molecular weight of 6,500. Partially purified enterocin EL1 added to sensitive cells of *Listeria ivanovii* was bactericidal; however, the bacteriocin did not inhibit the producer strain L1. Genetic determinants for bacteriocin production and immunity do not appear to be plasmid-borne.

### EFFECT OF TEMPERATURE, SALT AND pH ON GROWTH INHIBITION OF *LISTERIA MONOCYTOGENES* BY SODIUM POLYPHOSPHATE

O. Joseph Scullen,\* Biological Laboratory Technician, and Laura L. Zaika, USDA, ARS, ERRC, 600 East Mermaid Lane, Philadelphia, PA 19118

The effect of the multifunctional food additive, sodium polyphosphate (SPP), average chain length = 13, in combination with temperature (4, 12, 19°C), NaCl (0.5, 2.5, 4.5%) and initial pH (5, 6, 7) on aerobic growth of *Listeria monocytogenes* was evaluated. The experiments were done in Brain Heart Infusion medium containing 0, 0.1, 0.3, 0.5 or 1.0% SPP. Growth curves were fitted from plate count data using the Gompertz equation. Sodium polyphosphate exerted a bacteriostatic effect on Lm. Growth inhibition by SPP increased with decreasing temperature, decreasing pH and increasing NaCl concentration. At 19 and 12°C *L. monocytogenes* grew in the presence of 1.0% SPP even at the highest NaCl levels in media of pH 7. At 4°C, growth was restricted to salt levels  $\leq 0.5\%$  SPP. In pH 5 media, growth was prevented by 0.3, 0.1 and 0.0% SPP at 19, 12 and 4°C, respectively.

### EVALUATION OF DIFFERENT PHOSPHATES TO CONTROL MICROBIAL GROWTH IN MEAT PRODUCTS

Susan S. Sumner,\* Assistant Professor, Lisa M. Flores, Dianne Peters and Roger Mandigo, University of Nebraska-Lincoln, 356 Food Industry Building, Lincoln, NE 68583-0919

The efficacy of two different phosphate blends to inhibit the growth of foodborne pathogens in meat products was determined. The following products and microorganisms were tested: ground beef — *Escherichia coli* O157:H7 and *Listeria monocytogenes*; linked smoked sausage — *Salmonella typhimurium* and *L. monocytogenes*; cured smoked ham — *S. typhimurium* and *L. monocytogenes*; and fresh pork sausage — *E. coli* O157:H7 and *S. typhimurium*. All the products were prepared with 0.5% phosphate, individually inoculated with the challenge microorganism ( $10^3$  CFU/g), and stored at either 4, 12, 20 or 35°C. There was minimal to no effect of the phosphate blends on the growth of *L. monocytogenes*. At 4°C, *E. coli* O157:H7 decreased in ground beef and fresh pork sausage and at 12, 20 and 35°C, *E. coli* O157:H7 growth was inhibited 1-2 logs. *Salmonella typhimurium* demonstrated a 48% and 65% reduction at 4°C in linked sausage and ham, respectively. One phosphate blend in combination with the fresh pork sausage spice mixture (2% sodium chloride, 0.2% sage and 0.2% white pepper) was able to inhibit sporulation of *Clostridium sporogenes* at 25 and 35°C. These results indicate that phosphate blends can be used in certain meat products to delay the growth of foodborne pathogens.

### INHIBITORY ACTIVITY OF CAFFEIC ACID AGAINST *CLOSTRIDIUM BOTULINUM* SPORES

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Caffeic acid (CA) is a hydroxycinnamic acid derivative that is widely distributed among higher plants. While CA has been demonstrated to be antibacterial, antifungal and antiviral, little information exists on its activity against foodborne bacterial pathogens. As such, CA was tested for activity *Clostridium botulinum* spores in botulinum assay medium at 32°C. At 0.78 mM, CA inhibited germination while 3.25 mM were required to render spores non-viable. Caffeic acid concentrations  $\geq 50$  mM delayed toxinogenesis for 48 h and reduced spore 80°C thermal resistance. Two-tenths and  $>5.0$  mM CA were antimetabolite in commercial meat broths for 4 and 8 days, respectively. These data suggest that caffeic acid and perhaps other hydroxycinnamic acid derivatives might have potential for controlling growth of *C. botulinum*, and reducing thermal processing requirements.

### ANTIMICROBIAL EFFECT OF SODIUM LACTATE, TRI-SODIUM PHOSPHATE, AND SODIUM GLUTAMATE MONOHYDRATE PRETREATMENTS IN COMBINATION WITH ORGANIC ACIDS ON *ESCHERICHIA COLI* O157:H7

Peggy Wixom,\* Graduate Assistant, and J. S. Dickson, 2312 Food Sciences Building, Iowa State University, Ames, IA 50011

In an attempt to remove bacterial contamination during the slaughtering process, organic sprays have been implemented. In this study, the survival of *Escherichia coli* O157:H7 on beef tissue surface following sodium pretreatment and organic acid was examined. At room temperature, sodium lactate, tri-sodium phosphate and sodium glutamate monohydrate, at concentrations of 1%, 2% and 3%, were evaluated in combination with lactic acid, acetic acid and citric acid, at 1% and 2% concentrations. Selected pretreatment-treatment combinations were then further tested at 52°C. Colony counts of the bacteria were determined using trypticase soy agar and MacConkey's agar with sorbitol under aerobic conditions. Although there was bacterial reduction with all pretreatment-treatment combinations, a comparison of the pre-treatments showed the greatest overall effect was sodium lactate with a 0.8 log<sub>10</sub> reduction at 52°C. This identifies a potentially useful pretreatment spray for the postevisceration slaughtering process.

### MICROBIOLOGICAL SHELF-LIFE STABILITY OF TEXTURED SUPRO™ GRANULES

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Rehydrated isolated soy protein (Textured Supro™ Granules) was stored at 5, 10, 15, 20 and 25°C for 1 to 7 days and microbiologically evaluated. As expected, as the temperature and sampling time increased, so did the bacterial counts (e.g., psychrotrophic and mesophilic aerobic bacteria). Microbiological spoilage ( $10^6$  CFU/g) of the hydrated protein granules stored at 5, 10, 15, 20 and 25°C occurred at 4, 3, 2, 1 and  $<1$  days, respectively. The spoilage microorganisms were comprised mainly of gram-negative psychrotrophic bacteria (e.g., *Serratia* spp., *Enterobacter* spp. and *Pseudomonas* spp.).

### SHELF-LIFE AND MICROBIAL ECOLOGY OF PRECOOKED POULTRY STORED UNDER MODIFIED ATMOSPHERE AT 4°C

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Modified atmosphere packaged refrigerated ready-to-eat foods are becoming increasingly popular in North America. Little data is available on the dominant spoilage microbiota of these products. Characterization of the major spoilage organisms is important to predicting the quality and safety of the final product. The microbiota of oven-roasted poultry cuts (pH 6.6), packaged under 40:60 CO<sub>2</sub>:N<sub>2</sub> and stored at 4°C, was followed for a period of 7 weeks for three separate trials. Random samples were analyzed on a weekly basis for a number of microbial populations. Populations of all microbial populations tested were less than 100 CFU/g for the first 3 weeks of storage. Aerobic and anaerobic spores, and yeasts and molds were not

detected throughout the storage period. Presumptive lactic acid bacteria, aerobic, anaerobic and psychrotrophic plate counts were similar and increased to  $10^5$ - $10^6$  CFU/g by the 5th week and to  $10^7$ - $10^8$  CFU/g by the 7th week of storage. The product was acceptable to an untrained sensory panel throughout the storage period and showed no visible signs of deterioration. Representative colonies were selected from plates of the highest dilution for psychrotrophs, aerobes and anaerobes. Biochemical analysis indicated that the microbiota consisted of gram-positive bacteria, many of which were classified as *Carnobacterium* spp.

#### EFFECT OF WATER ACTIVITY AND HUMECTANT IDENTITY ON THE GROWTH KINETICS OF *ESCHERICHIA COLI* O157:H7

Robert L. Buchanan,\* Research Leader, and Lori K. Bagi, USDA, ARS, ERRC, 600 East Mermaid Lane, Philadelphia, PA 19118

The effect of water activity ( $a_w$ ) and humectant identity (sorbitol, mannitol and NaCl) on the growth kinetics of a three strain mixture of *Escherichia coli* O157:H7 was assessed using Brain Heart Infusion adjusted to 3 pH levels (5, 6 and 7) and incubated at 3 temperatures (12, 19 and 28°C). Media 50 ml containing 50, 100, 150 or 200 g/l sorbitol or mannitol ( $a_w = 0.983 - 0.968$ ) were dispensed in 250-ml flasks, inoculated with 1000 CFU/ml, incubated with agitation, and periodically assayed for viable counts. Growth curves were fitted using the Gompertz equation and the growth kinetics compared with previously developed models where sodium chloride (NaCl) was the humectant. The response to mannitol and sorbitol was generally comparable. At 28°C, the "time to a 1,000-fold increase in population" was linearly related to  $a_w$ , though there tended to be some deviation from linearity at the lowest pH and  $a_w$ . The microorganism was more resistant to the sugar alcohols than NaCl. At the lower incubation temperatures, the responses to elevated levels of sorbitol and mannitol were more variable and the differential between these humectants and NaCl was less distinct. The data indicate that humectant identity can impact the effect that water activity has on the growth of *E. coli* O157:H7.

#### RESISTANCE OF ACID ADAPTED SALMONELLAE TO ORGANIC ACID RINSES ON BEEF

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Four strains of *Salmonella*, including three cattle isolates and an ATCC strain, were adapted to growth in acidic conditions by sequential transfer in tryptic soy broth with reduced pH values. The cultures were transferred until good growth (approx.  $\log_{10}$  7 CFU/ml) was obtained within 24 h at 37°C at pH 5.0. Lean beef tissue was inoculated by immersion into either acid adapted or the homologous parent strain of each bacterium. The inoculated tissue was washed for 10 sec. in either distilled water or lactic acid at either 1.5° or 3.0° at 23°C or 55°C. The reductions in bacterial population were compared between the parent and acid adapted strains to determine if the acid adapted strains were more resistant to the organic acid rinses. Acid adapted strains had either equal or greater sensitivity to organic acid rinses than their homologous parent strains, indicating that acid adaptation did not result in bacteria, which were resistant to organic acid rinses.

#### SURVIVAL OF *E. COLI* O157:H7 IN REFRIGERATED AND FROZEN LOW FAT GROUND BEEF AND THERMAL INACTIVATION BY MICROWAVE ENERGY

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The objective of this project was to determine the survival of *Escherichia coli* O157:H7 in low fat ground beef during microwave cooking and in low fat ground beef held under different storage conditions. Ground beef at 4 lean levels, 80, 90, 94 and 96% lean, was irradiated at 5 kGy. Samples were inoculated with  $10^3$  CFU/g. Samples stored at -20°C for 56 days revealed constant to 0.5 log decrease of *E. coli* O157:H7 populations in all lean levels. For samples held at 4°C, results showed that *E. coli* O157:H7 counts decreased in 80, 90 and 94% lean, but counts remained constant in 96% lean. Additional ground beef at 3 lean levels, 80, 90 and 96% lean, was irradiated at 5 kGy. The ground beef was inoculated with  $10^3$  CFU/g and formed into 225 g meat loaves. Duplicate loaves at each lean level were cooked in a microwave at full power (900 W, 2,450 MHz) to achieve a final temperature

of 50°C or 55°C in each of 6 portions/areas within each loaf. Each portion was plated separately using phenol red sorbitol agar with 1% pyruvate. Meat loaves —cooked according to a microwave recipe — which reached a final temperature of 75°C, had no survivors at any lean level. These research results indicate that if consumers follow standard microwave guidelines, ground beef would be properly cooked.

#### THE FATE OF *LISTERIA MONOCYTOGENES* AND *CLOSTRIDIUM BOTULINUM* IN MINIMALLY-PROCESSED PACKAGED VEGETABLES

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The minimally-processed fresh-cut produce industry is a rapidly expanding one offering consumer size packages of fresh, nutritious product. A modified atmosphere (MA) usually develops very quickly in these packaged products due to product respiration. Since the microbiological safety of these types of products is not known, we have undertaken a large study to address this issue in minimally-processed, modified atmosphere packaged (MAP) vegetables.

Challenge studies of MAP vegetable products were conducted using both *Listeria monocytogenes* and *Clostridium botulinum*. In all products, oxygen levels declined at both 4 and 10°C, while CO<sub>2</sub> levels increased. *Listeria monocytogenes* was unable to multiply on packaged freshly cut onions stored at 4°C for 21 days. However, at 10°C, *L. monocytogenes* increased by almost 2 logs within 2 weeks. Proteolytic *C. botulinum* strains were not able to grow and produce toxin at 25°C within 3 days, however, toxin was detected on day 6. On butternut squash stored at 4 and 10°C, *L. monocytogenes* increased in number by approximately 2.5 and 6.5 logs respectively, over a 14-day storage period. With nonproteolytic strains of *C. botulinum*, toxin was detected on squash stored at 5° and 10°C on day 21 and 7, respectively. Results to date demonstrate the importance of strict temperature control to ensure the safety of these products.

#### USE OF TIME-TEMPERATURE INDICATOR TO MONITOR THE SHELF-LIFE OF PACKAGED FRESH CATFISH

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Full-history time-temperature indicators (TTI) are becoming increasingly noticeable by food manufacturers, market managers and consumers to use for monitoring food quality during distribution and retailing. The objective of this study was to examine consumer readable TTIs to determine the microbial quality changes of packaged channel catfish. Both skinned and skin-on catfish fillets, held in either film overwrapping or vacuum skin packaging, were stored at 4°C. A commercial available polymer-based TTI was used to monitor the shelf-life of packaged fillets. The samples were evaluated for Hunter color values (L, "a," "b") and psychrotrophic plate count at a 4-day interval for 16 days. Results showed that one model of the TTI with a similar activation energy for deterioration can be reliably used as end of shelf-life indicator for catfish. Yet by using instrumental measurement, psychrotrophic plate count of fillet could be predicted by the TTI response (total color difference) obtained under storage temperature condition.

#### RECOVERY OF *ARCOBACTER* FROM BROILER CARCASSES

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*Arco bacter* spp. has been shown to be a causative agent in human disease. It has been isolated from the environment (sewage, water) and animals (cattle, pigs, horses, monkeys, ostriches). One study reports *Arco bacter* spp. isolations from poultry meat. *Arco bacter* spp. is related to the genus *Campylobacter* and differs primarily in its ability to grow aerobically. Our study showed that *Arco bacter* spp. are frequently, but not always, isolated from boilers when *Campylobacter* spp. are present. Of 28 carcasses tested, 82% were positive for both species, 10.8% for *Campylobacter* spp. only, 3.6% for *Arco bacter* spp. only, and 3.6% were negative. There were skips in isolations from consecutive whole carcass rinses, indicating low levels of *Arco bacter* spp. on broilers and/or sporadic recovery probably due to imperfect isolation techniques. The epidemiological association of such isolations from poultry carcasses and human disease is still unproven.



## MONOCLONAL ANTIBODY FOR RAPID DETECTION OF *CLOSTRIDIUM BOTULINUM* TOXIN TYPE B

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The threat of botulism to human life is a major food safety concern for FDA. Seven immunologically distinct toxin types are synthesized by different strains. Types A, B, E and F affect man. Hybridoma cells producing monoclonal antibody against Type B *Clostridium botulinum* toxin were screened for antibody production, subcultured and cloned by limiting dilutions. Several clones were obtained as secretors of monoclonal antibodies against Type B toxins. Monoclonal antibodies in combination with ELISA were used to detect Type B *C. botulinum* toxin in culture fluids and food products at approximately 10 ML/D/ml in 6-8h. No cross reactions with other botulinum toxin types (A, C, D, E, F and G) as well as *Clostridium tetani*, *Clostridium perfringens*, *Clostridium novyi* and *Clostridium sporogenes* were observed. The ELISA technique in combination with the monoclonal antibody provide a rapid, sensitive and accurate assay for the detection of Type B botulinum toxin.

## SUSCEPTIBILITY OF *LISTERIA* sp. TO CELL BOUND PEDIOCIN ACh IN BHI TURKEY, TURKEY FRANK SLURRIES, AND ON CHICKEN BREAST MEAT

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The bacteriocin, pediocin ACh, was bound to the heat killed cells of the producer strain, and freeze dried. Food grade acids were used to release the pediocin from the cells, at levels that were not inhibitory to the target species. D-gluconic acid lactone was chosen to be used in the following experiments. The inhibitory spectrum of the preparation was tested against 96 species and strains of *Listeria* with 93.8% of the organisms tested being found to be susceptible to pediocin ACh. Of 66 strains of *Listeria monocytogenes* tested for susceptibility, 89.4% were susceptible to the preparation, with no one serotype showing more resistance than the others. Also included in these strains were several clinical outbreak isolates. At 37°C, growth in BHI broth of 10<sup>5</sup> CFU/ml of *L. monocytogenes* could be inhibited by 800 AU/ml of the preparation for up to 5 days. Serotypes 4b, 1/2a, 1/2b and 1/2c were used in these experiments with no difference in response being noted. At 8°C in BHI, growth of 10<sup>5</sup> CFU/ml of *L. monocytogenes* was controlled by 2400 AU/ml for a period of 25 days. At 8°C, over 28 days, in a slurry made of turkey franks, levels of 800 AU/ml were effective in controlling 10<sup>5</sup> CFU/ml of *L. monocytogenes*. When surface inoculated with 10<sup>5</sup> CFU/g of *L. monocytogenes* on whole muscle chicken breast at 8°C, there was a restriction in growth of 4 logs over 20 days at the 2,400 AU/g level compared to the control, with final counts of 8.3x10<sup>6</sup> and 5.0x10<sup>10</sup> CFU/g, respectively.

## THE FATE OF *LISTERIA MONOCYTOGENES* DURING THE MANUFACTURE OF MANCHEGO CHEESE WITH BACTERIOICIN-PRODUCING LACTIC ACID BACTERIA AND COMMERCIAL LACTIC STARTERS

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Manchego cheese was manufactured from ewes ray milk inoculated with *Listeria monocytogenes* in duplicate trials, each consisting of three vats. Milk in vat 1 was inoculated with 1% of MAO 11 (Texel), a commercial mesophilic lactic starter; milk in vat 2 was inoculated with 1% of INIA 4, a bacteriocin-producing *Enterococcus* sp.; milk in vat 3 was inoculated with 1% of MAO 11 and 1% of INIA 4. Mean log count of *L. monocytogenes* in milk after inoculation (0h) was 5.02. Log count of *L. monocytogenes* in curd (2 h) was 4.45, <2.000 and 2.15 for vats 1, 2 and 3. Bacteriocin in curd (in equivalents nisine, mg/g) was 0, 0.075 and 0.062 in vats 1, 2 and 3; curd pH was 6.39, 6.69, respectively. In fresh cheese (24 h), log count of *L. monocytogenes* was 4.83 in vat and 1 and <2.00 in vats 2 and 3. Bacteriocin in fresh cheese was 0, 1.421 and 0.460 in vats 1, 2 and 3; pH was 4.96, 6.47

and 5.05. Thus, *L. monocytogenes* may be inhibited by bacteriocin-producing lactic acid bacteria in Manchego cheese without perturbing lactic acid production.

## MICROBIAL CHANGES OF OSMOTICALLY DEHYDRATED GREEN BEANS COUPLED WITH MODIFIED ATMOSPHERE PACKAGING STORED AT 10°C

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Osmotic dehydration (OD) is an innovative method for preparation of minimally processed vegetables which reduces respiration rate and improves shelf-life. Microbial quality and safety of beans processed using OD had not been studied. Therefore, this study was undertaken to determine the growth and survival of spoilage and pathogenic bacteria on OD beans. Beans were osmotically dehydrated by immersion in saturated NaCl solution, followed by sanitizing in 200 ppm chlorine solution for 10 s before packaging. Beans were vacuum-packaged in PD961 EZ film (Cryovac). Controls were packaged in films under ambient atmosphere. Beans were inoculated with 10,000 CFU/g of *Listeria monocytogenes* or *Salmonella typhimurium* to determine growth and survival characteristics in the product. Aerobic plate counts were not significantly (p>0.05) affected by OD. However, pH was on pH unit lower in osmotically dehydrated beans probably as a result of the higher lactic acid population in OD beans. The shelf-life of green beans prepared using OD and without OD under air of vacuum packaging was approximately 12 days. *Listeria* and *Salmonella* were not detected on OD beans during storage.

## MOLD CONTENT OF STORED POPCORN

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The purpose of this study was to determine the mold content of samples of commercial popcorn in different packages, and the effect of high temperature (30-35°C) and high humidity (85-87%) on the development of mold in popcorn stored in different packages. Total mold infection levels were determined for microwave (yellow) popcorn samples, white and yellow popcorn in plastic bags or other non-microwave packages. The total mold infected kernels were rather low in microwave (7.5%) packages, and in yellow non-microwave (7.6%) packages, but somewhat higher in white non-microwave (16.3%) packages. Of the molds found, *Fusarium* species predominated in the microwave popcorn (58.8%) and white non-microwave popcorn (70.6%), but not in the yellow non-microwave popcorn (33.3%). Of the *Fusarium* species found, *Fusarium molitiforme* was the most frequently found species in microwave (70.6%), yellow (73.9%) and white (70%) non-microwave packages and specialty (colored) popcorn (91.7%). Other *Fusarium* species found included, *Fusarium proliferatum*, *Fusarium subglutinans*, *Fusarium semitectum* and *Fusarium anthophilum*. Percent of mold infected kernels increased when stored popcorn was exposed to high temperatures and high humidities, but not if the popcorn was protected in a sealed container or plastic bag.

## EFFECT OF DRY MILLING ON *FUSARIUM* COUNTS AND FUMONISINS IN CORN

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The incidence of *Fusarium* species and the toxin fumonisin found in food grade corn and its fractions from a commercial dry mill were surveyed. A weekly sample (for a period of 12 weeks), of the whole corn, germ, bran, flaking grits and flour were analyzed for *Fusarium* counts and fumonisins. Infection of whole corn by *Fusarium* species ranged from 10 to 25% infected kernels. *Fusarium* counts in the corn fractions were lowest in the flaking grits (all <100 CFU/g) and the flour (<100 - 2,700 CFU/g). *Fusarium* counts were higher in the bran (<100 - 64,000 CFU/g) and in the germ (<100 - 16,000 CFU/g). Total fumonisin content in the whole corn ranged from <0.1 µg/g to 3.5 µg/g. Total fumonisin content in the corn fractions were low in both the flaking grits (all <0.5 µg/g and flour (all <0.5 µg/g), but higher in the bran (1.5 - 3.2 µg/g) and in the germ (0.2 - 2.0 µg/g).

### ISOLATION OF THE ZEARELENONE-PRODUCING STRAINS FROM AGRICULTURAL PRODUCTS IN SOUTHERN KOREA

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Zearalenone-producing strains were isolated from agricultural products in southern Korea. Various samples such as rice (60), barley (52), soybean (45), peanut (33), corn (32), maeju (27), unhulled rice (UR, 40), unhulled barley (UB, 54), and soil (32) were collected. From 375 samples, 302 *Fusarium* strains were isolated. The isolated strains were cultured for 14 days at 30°C in rice medium, and then screened for zearalenone-producing strains by TLC and HPLC. The results were that 29 isolates [rice (3), maeju (2), corn (1), barley (5), soil (4), peanut (3), soybean (4), U.B. (5), U.R. (2)] were identified as zearalenone-producing strains by TLC analysis while 19 isolates [rice (2), maeju (1), corn (1), soil (3), soybean (2), U.B. (3), U.R. (3)] were detected by HPLC method. An ELISA method was applied to determine the zearalenone-producing abilities of these isolated strains. Among 19 strains, 3 strains (R-5, C-46, S-134) produced more than 50 ng/ml of zearalenone. The above results imply that agricultural products in southern Korea were contaminated by zearalenone producing fungi, therefore further studies are required to accumulate more data.

### INHIBITION OF PHOSPHATE ON MOLD GROWTH AND MYCOTOXIN PRODUCTION (T-2 TOXIN, ZEARELENONE)

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For the control of hazardous mycotoxin and mycotoxigenic fungi on cereal and foodstuff, sodium acid polyphosphate was examined for the effects on mold growth and *Fusarium* toxin (T-2 toxin and zearalenone) production. We inoculated *Fusarium sporotrichoides* M-1-1 and *Fusarium graminearum* w-5 on PDA medium and rice medium, and cultured at 28°C for 14 days. Both used strains significantly inhibited the growth on PDA medium, and the same tendency of mold growth was shown on natural rice medium with a 1% sodium acid polyphosphate addition. T-2 toxin and zearalenone production were also inhibited to an extent of 89%, 84% on natural rice medium, respectively.

### IMMUNOLocalIZATION OF AFLATOXIN B<sub>1</sub> IN LIVER OF CHICK EMBRYO INTOXICATED WITH AFLATOXIN B<sub>1</sub>

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Aflatoxicosis is a serious human health concern. The present studies describe the accumulation of aflatoxin B<sub>1</sub> in liver of chick embryo intoxicated with aflatoxin B<sub>1</sub>. A single dose of aflatoxin B<sub>1</sub> (0.05 µg/egg) was injected into the yolk sac of 12-day-old eggs and livers were obtained at 3, 6 and 8 days later. By immunohistochemical method, we found that aflatoxin B<sub>1</sub> was barely detectable in liver at 3 days post-injection, but 6 days post-injection aflatoxin B<sub>1</sub> began to appear in the Kupffer cells. At 8 days post-injection, aflatoxin B<sub>1</sub> was widely disseminated throughout the tissue, especially in Kupffer cells, in endothelial cells, and in perisinusoid hepatocytes. This study helps explain the etiology and development of aflatoxicosis. Through such understanding we hope to reduce the foodborne disease of aflatoxicosis.

### THE MYCOFLORA AND MYCOTOXIN-PRODUCING POTENTIAL OF FUNGI FROM FOODS IN BURUNDI

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The mycoflora of food products in Burundi was isolated and identified with emphasis on species of *Aspergillus*, *Fusarium* and *Penicillium*. Randomly selected isolates from the most commonly occurring species of the three genera were also examined for their ability to produce mycotoxins. *Fusarium moniliforme* was the most predominant fungus isolated from corn and sorghum. The predominant fungi from dried beans were *Fusarium semitectum* and *Fusarium equiseti*. *Fusarium semitectum* was also the most predominant species isolated from peanuts. Peas were predominantly

infected with *Aspergillus ochraceus* and *Aspergillus wentii*. A variety of *Aspergillus* species including *A. flavus* and *A. niger* were isolated from dried non-salted fish. Only 32% of *A. flavus* isolates produced aflatoxins whereas 67% of the same isolates produced cyclopiazonic acid (CPA). However, no aflatoxin was detected in the food products. Almost 50% of *Aspergillus oryzae* and *Aspergillus tamarii* isolates also produced CPA. The presence of these mycotoxin-producing fungi in foods pose a potential threat to human health in Burundi.

### APPLICATION OF IMMUNOHISTOCHEMICAL TECHNIQUE TO VISUALIZE ZEARELENONE FORMATION OF *FUSARIUM GREAMINEARUM*

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An enzyme-linked immunohistochemical technique (ELICT) was applied to visualize the zearalenone formation within hyphae. The mycelium of *Fusarium graminearum*, a zearalenone producing strain, was used as a model system for developing the immunohistochemical procedure. Twenty milliliters RYA (rice 5%, yeast extract 0.1%, agar 1.5%) medium was dispensed into disposable petri dish and 50 µl microconidial solution (5 x 10<sup>6</sup> conidia/ml) was inoculated onto plates and the inoculated media were incubated at 30°C. Before the immunohistochemical test for visualization of zearalenone deposits within the strain, contents of zearalenone in media cultured for 3, 9 and 15 days were analyzed. At day 3, 0 ng/cm<sup>2</sup> zearalenone, at day 9, 14 ng/cm<sup>2</sup> and at day 15, 24.5 ng/cm<sup>2</sup> were detected. The ELICT assays were consistent with the ELISA results. Mycelia obtained from 3-day-old cultures did not react but 9 and 15-day old mycelia developed dark brown granules, and manifested zearalenone deposits in host tissue. These studies demonstrate the zearalenone within the hyphae of *F. graminearum*, characterizing the production of aflatoxin, a serious human health concern.

### USE OF TECRA® UNIQUE™ FOR THE DETECTION OF SALMONELLA IN A RANGE OF FOOD PRODUCTS WITHIN 22 HOURS

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The TECRA® unique™ test for *Salmonella* recognizes all species of *Salmonella*, both motile and non-motile. It is a self-contained system comprising an antibody-coated dipstick and a module of six foil-sealed tubes. Over 300 naturally-contaminated and 50 artificially inoculated food samples were tested using unique, and the Australian Standard method and MIRINZ Method for Microbiological Examination of Food. The unique test showed excellent correlation with these standard methods in a wide variety of samples containing *Salmonella*. An overall false positive rate of <1% was recorded. The unique test also proved to be a very convenient and fast method for screening for *Salmonella*. All reagents are contained ready-to-use in the module, and the transfer of the dipstick is a simple operation. Most current methods used to screen for *Salmonella* in food involve toxic, selective media, but the use of unique's antibody-coated dipstick obviates the need for these. The unique test also improves productivity in the laboratory, as actual "hands-on" time is minimal and unique's applicability to a very side range of food products and environmental samples makes it a very versatile test.

### A PREDICTIVE MODEL WITH IMPROVED STATISTICAL ANALYSIS OF THE INTERACTIVE EFFECTS OF FACTORS AFFECTING THE GROWTH OF *STAPHYLOCOCCUS AUREUS* 196E

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Growth of pathogenic bacteria in foods is affected by several factors which may interact to enhance or inhibit microbial growth. Models to predict the growth of *Staphylococcus aureus* 196E in media were developed using a modified Gompertz function and response surface methodology. A statistical model was used to determine the significance of interactions among time, incubation temperature, pH and NaCl concentration on growth. *Staphylococcus aureus* 196E was inoculated into Brain Heart Infusion broth formulated with either 0.5, 4.5 or 8.5% NaCl, adjusted to pH 5.0, 6.0 or 7.0, and incubated aerobically at 12, 20 or 28°C. Several interactive relationships between time, temperature, pH and NaCl concentration were significant. Predicted responses to multiple factor interactions can be displayed with contour plots.

The methodology can provide important information to food microbiologists such as predictions of lag phase duration and maximum population density and confidence intervals for growth parameters.

#### **AUTOMATED DETECTION OF FOODBORNE PATHOGENS USING THE TECRA® OPUS® SYSTEM**

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**TECRA® OPUS®** is a new, automated immunoassay system for the rapid detection of foodborne pathogens. The system incorporates novel assay-specific test modules, each of which contains all the reagents necessary to perform one ELISA. The patented **OPUS** technology is based on a fluorogenic endpoint. The machine has the capacity to process around 160 samples per day and requires minimal "hands-on" time. The **TECRA OPUS Salmonella** assay recognizes all species of *Salmonella*, both motile and non-motile. This assay was evaluated on a range of food and environmental samples using two different methods of enrichment. One method consisted of the more conventional 48-h pre-enrichment and selective enrichment procedures, whilst the other involved the use of the **TECRA 24-h Immunocapture™** procedure in place of the selective enrichment step. Both enrichment methods were found to be compatible with the **OPUS** immunoassay system and thus, provided maximum flexibility for the food testing laboratory. The **OPUS Listeria** assay detects all species and serotypes of *Listeria* with a sensitivity of at least  $10^5$  cells/ml. An evaluation of this method was undertaken to compare its performance with that of standard enrichment and plating techniques. The samples included dairy products, meats and environmental swabs. The results showed the **TECRA OPUS Listeria** assay to be at least as sensitive as the standard method.

#### **AGGLUTINATION BEHAVIOR OF LACTIC STARTER CULTURES**

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Twenty-seven commercial starter cultures that were initially grown in skim milk, had different subsequent subculturing (2) and then were inoculated into pasteurized skim milk (2,000 ml in a graduated cylinder) at 5% inoculum level. Inoculated skim milk samples were incubated in a water bath at 31°C for 5 h. At the end of the incubation period, samples were taken from the top and bottom of each graduated cylinder and analyzed for pH and for agglutination response using direct microscopic examination. The proteolytic activity of selected agglutinated (4) and non-agglutinated (2) cultures were tested using trinitrobenzenesulfonic acid (TNBS).

Ten cultures (37%) showed severe agglutination and 15 cultures (56%) had moderate agglutination. Agglutinating cultures showed less proteolytic activity than non-agglutinating cultures ( $p < 0.11$ ).

Agglutination is a severe problem among Jordanian starter cultures. New means for production of active and stable starter cultures should be investigated.

#### **EFFECTS OF PACKAGING SYSTEM ON LACTATE TREATED TILAPIA FILLET STORED AT 4°C**

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Tilapia (*Oreochromis* spp.) fillets were dipped in 8% sodium lactate or a mixture of 4% sodium lactate and 4% potassium lactate for 2 min. Treated fillets were either overwrapped, vacuum skin packaged or modified atmosphere (70% CO<sub>2</sub> and 30% N<sub>2</sub>) packaged and then stored at 4°C for 16 days. Psychrotrophic plate count, total anaerobic plate count, pH, and Hunter color value (L, "a," "b") of fillets held in modified atmosphere packaging had the lowest ( $p < 0.05$ ) psychrotrophic and anaerobic plate counts, followed by those held in vacuum skin packaging and overwrapping, as compared to control samples, regardless of treatment. Surface pH values of both sodium lactate and a mixture of sodium and potassium lactate treated fillets were significantly lower than that of control, however, no significantly lower than that of control. No significant differences in Hunter color values among samples were found regardless of treatment and packaging method.

## **A SYMPOSIUM ON RISK MANAGEMENT**

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### **RISK ASSESSMENT: The Risk Analysis Approach**

#### **ISSUES IN THE ASSESSMENT OF FOOD SAFETY RISKS**

##### **INFECTIOUS OOSE AND SUSCEPTIBLE POPULATIONS**

Dr. Michael van Schothorst, Nestlé, Av Nestlé 55, Vevey, Switzerland

Foodborne pathogens are widespread in nature. They can be found, with varying frequencies, in primary agricultural and aquatic products, food factories, food preparation areas and household kitchens. Man is thus constantly exposed to them. Fortunately, this rarely results in clinical illness. The human response depends on the type of food, the numbers and pathogenicity of the incriminated microorganism and on the susceptibility of the host. Age, immune status, nutritional status, underlying diseases, stress and use of medication are among the factors determining susceptibility. Numbers of potential pathogens which are mostly harmless for the "normal" individual may cause illness in susceptible persons. The risk factors determining the dose response relationships need to be elucidated and if possible, quantified. Increased susceptibility can be found amongst the very young and very old, the diseased, the malnourished and some other subgroups of the human population. These subgroups have to be considered separately in hazard analysis, risk assessment and risk management exercises.

##### **THE ROLE OF EPIDEMIOLOGY IN ESTIMATING RISK AND RISK EXPOSURE**

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Epidemiologic studies of foodborne disease provide important information for estimating risk. Foodborne hazards are identified during investigations of outbreaks of foodborne disease. Outbreak investigations also provide information on risk factors for infection and indicators of severity of disease. Prospective studies of sporadic foodborne diseases provide data on infection rates, attributable fractions, and other information useful for characterizing risk. Clinical laboratory based surveillance for infections with foodborne microorganisms provides data on disease trends and characteristics of foodborne pathogens.

##### **THERMAL INACTIVATION KINETICS FROM CONTINUOUS FLOW AND BATCH HEATING SYSTEMS**

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Thermal inactivation of *Listeria innocua* (at 65 to 70°C), *Bacillus cereus* spores (at 99 to 107°C) and *Bacillus stearothermophilus* spores (at 128.5 to 139°C) was monitored under continuous flow conditions and compared to results from the capillary tube (batch) heating method. A laboratory scale pasteurizer was used to generate kinetic data under isothermal, continuous flow conditions. Inactivation was monitored by sampling at various locations along the hold tubes.  $z_p$ -Values from the batch and continuous systems were significantly different for *L. innocua* and *B. stearothermophilus* spores but not for *B. cereus* spores. The challenge of kinetic evaluation in continuous heating systems will be discussed. The data illustrate that the use of batch generated kinetic data to design continuous flow processes may not be accurate.

##### **SHELF-LIFE EXTENSION OF COTTAGE CHEESE BY DISSOLVED CO<sub>2</sub>**

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The inhibitory effect of carbon dioxide on many gram-negative microorganisms has been used in modified atmosphere packaging (MAP) to extend the shelf-life of refrigerated foods. However, MAP has not been broadly applied to liquid or semi-liquid foods because its packaging does not lend itself to conventional gas flushing. We have developed a system for creamed cottage cheese where CO<sub>2</sub> is added directly to the cream dressing prior to mixing with the curd. After mixing, the cottage cheese is packaged in tubs

with either a high barrier shrink wrap or heat sealed membrane seal. Amounts of carbon dioxide which are below sensory detection inhibit spoilage organisms and increase shelf-life from approximately 21 days to more than 45 days at 7.5°C. CO<sub>2</sub> also enhances safety by inhibiting *Listeria monocytogenes* and does not enhance the growth of *Clostridia sporogenes*. This technology has been tested in a commercial cottage cheese plant and is currently being adopted for routine use. Current work involves extending the concept to other fluid dairy products.

#### BACTERIA OF CONCERN IN EXTENDED SHELF-LIFE MILK

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Processing temperatures below 120°C will not provide an acceptable ESL product consistently. Milk heated less than 120°C had a lower microbial count immediately after processing, but counts increased sporadically during storage at 7°C, suggesting many of the bacteria were heat shocked and viability was unpredictable. Milk processed at 100°C had high coliform and psychrotrophic counts after 15 days of storage at 7°C. Viable spore-forming bacilli were isolated from ESL milk processed between 120°C and 132°C. *Bacillus* species isolated included *Bacillus insolitus*, *Bacillus cereus/thuringiensis* and *Bacillus coagulans*. The proportion of containers in which these organisms grew during storage decreased with increasing processing temperature. Results suggest milk processed below 132°C could be unsafe, particularly if the product was temperature abused during storage. This study found that milk processed at 134°C for 4 s had little risk of residual microbial contamination, had an extended shelf-life (greater than 60 days at 7°C) compared to pasteurized milk, and had better flavor characteristics than UHT milk.

#### KNOWING AND CONTROLLING CHEESE PATHOGENS

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Improvements in the production, processing, distribution and storage of foods by both the food industry and the consumer have significantly diminished, but not eliminated, the likelihood of food-related illness. Due to the number, severity and costs of foodborne disease episodes, there is considerable interest in evaluating strategies to better characterize and control undesirable bacteria associated with food. This presentation will briefly highlight advances in the application of genomic fingerprinting for typing microbes and summarize progress using biopreservatives to control pathogenic and spoilage microbes.

#### DAIRY PRODUCT SAFETY SYSTEM (HACCP) DESIGNED SPECIFICALLY FOR THE INDUSTRY

J. Russell Bishop,\* Director, and Robert D. Byrne, WI Center for Dairy Research, 1605 Linden Drive, Madison, WI 53706

Hazard Analysis Critical Point (HACCP) program is a management tool that provides a logical and cost-effective basis for better decision making with respect to dairy product safety. A key advantage of the HACCP concept is that it enables dairy food manufacturing companies to move away from a philosophy of control based on testing to a preventive approach which identifies and control potential hazards in the manufacturing environment. HACCP is only a part of a Dairy Product Safety System. Any successful system must also include GMPs, potable water, plant hygiene and employee training. Without these in place as prerequisites, HACCP can never be successful. Critical points must be kept to an absolute minimum so that critical areas are properly highlighted and not confounded with less critical points in a process. The International Dairy Foods Association, in conjunction with CDR, has developed a Dairy Product HACCP program in order to take a leadership role in this area so that the industry and the federal government, along with state agencies, can agree on an acceptable system. This program includes model/generic HACCP plans for fluid milk, ice cream, yogurt, butter, cheddar cheese and mozzarella cheese. To complete this program, model programs are available in a computer software program which can be modified for each individual dairy operation.

## NATURAL ANTIMICROBIALS AND INHIBITORS FOR FOOD APPLICATIONS

Sponsored by the ILSI North American Technical Committee on Food Microbiology

#### BACTERIOCINS FOR LISTERIA CONTROL

Peter M. Muriana, Department of Food Science, Purdue University, W. Lafayette, IN

Foodborne outbreaks of listeriosis by *Listeria monocytogenes* have contributed to public consciousness about bacterial pathogens involved with foodborne disease. Major concerns with *L. monocytogenes* are its high fatality rate, wide distribution on raw products, growth at low temperatures, and its ability to establish itself in various food processing environments. These concerns have prompted the examination of novel approaches to combat its survival in foods, among which include the use of antimicrobial peptides or bacteriocins. Bacteriocins from lactic acid bacteria have received much attention because these microorganisms have a long history of safe use in foods either as starter cultures or as indigenous contaminants. Some bacteriocins are inhibitory to foodborne pathogens including *Listeria* and provide substantial reason for investigating their potential use in novel food preservation applications. Nisin is currently accepted worldwide and in the U.S., however, numerous other bacteriocins also have potential use in similar applications. Recent examples suggest that bacteriocins may contribute as an additional barrier in the "hurdle concept" of food safety.

#### POTENTIAL FOR USE OF BACTERIOCIN-PRODUCING LACTIC ACID BACTERIA FOR PRESERVATION OF MEATS

Lynn M. McMullen\* and Michael E. Stiles, University of Saskatchewan, Saskatchewan, Canada SK S7N 0W0

Bacterial spoilage and safety are major concerns in the marketing of raw and processed meats. With the use of modified atmosphere packaging (including vacuum-packaging) in which elevated levels of carbon dioxide are present, the prevailing microflora of meat is changed from aerobic, putrefactive bacteria to lactic acid bacteria. Some "new generation" convenience foods rely almost entirely on refrigeration for assurance of safety against growth of pathogenic bacteria. With the emergence of cold tolerant foodborne pathogens, it is desirable to increase the "hurdles" to pathogen growth. Lactic acid bacteria preserve meats by competitive exclusion of other microorganisms but they also produce inhibitory substances, including lactic and acetic acids and bacteriocins. Bacteriocins are naturally produced peptides that are antagonistic to other closely related bacteria. Although bacteriocins are expected to have a narrow range of antibacterial activity, nisin is a bacteriocin that is active against a relatively broad spectrum of gram-positive bacteria, including inhibition of the outgrowth of *Clostridium botulinum* spores. Nisin is not effective in meat systems; as a result, research on the lactic acid bacteria of meats is focused on the selection of lactic acid bacteria that do not cause meat spoilage and enhance product safety.

#### EFFICACY OF NATURALLY OCCURRING FOOD FLAVORS AS INHIBITORS OF FOODBORNE PATHOGENS

Bobby L. Bowles\* and Arthur J. Miller, Microbial Food Safety Unit, USDA/ARS Eastern Research Center, 600 East Mermaid Lane, Philadelphia, PA 19118

Although several naturally occurring plant components have been shown to be antibacterial and antifungal, only limited efforts have been made to exploit the antimicrobial properties of these agents in foods. As such, a number of approved naturally occurring flavor compounds were tested for activity against various foodborne pathogens to define their potential as antimicrobial agents. The compounds tested inhibited, to varying degrees, growth of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Shigella flexneri*, *Bacillus cereus*, *Aero-monas hydrophila* and proteolytic *Clostridium botulinum*. Aliphatic compounds were most active against gram-negatives, while aromatics were more effective against gram-positives. Pyruvaldehyde (0.39-25 mM) was the most active of 4 compounds tested against vegetative cells and *B. cereus* was the most sensitive bacterium. Six-tenths millimeter benzaldehyde, piperonal or vanillin inhibited *C. botulinum* spore germination, and 1.25 mM vanillin

rendered *C. botulinum* spores non-viable. One-hundred mM cinnamaldehyde or piperonal reduced *C. botulinum* spore thermal resistance and increased radiosensitivity. Similar activities were observed when combinations of cinnamaldehyde, benzaldehyde, pyruvaldehyde or piperonal were tested against *C. botulinum* spore thermal resistance. Diacetyl, benzaldehyde, pyruvaldehyde or piperonal reduced the thermal resistance of vegetative cells heated at 56°C for 30 min. These data indicate that naturally occurring flavor compounds, singly or in combination, may be employed to inhibit growth of a variety of foodborne pathogens, and to reduce thermal or irradiation processing requirements for some foods.

#### REGULATORY PERSPECTIVES ON THE USE OF BACTERIOCINS IN FOODS

F. Owen Fields, Ph.D., U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, HFS-207, 200 C Street, S.W., Washington, DC 20204

Antimicrobials, including bacteriocins, which are added to processed food must be: 1) approved as food additives by FDA; 2) generally recognized as safe (GRAS) by competent experts and therefore fall under the GRAS exemption to the food additive definition. Antimicrobials which are used on raw agricultural commodities are legally defined as pesticides and are therefore regulated by EPA. Because of their polypeptide nature, some bacteriocins may be eligible for status as generally recognized as safe ingredients.

#### USDA'S REGULATORY PERSPECTIVES ON THE USE OF BACTERIOCINS IN FOODS

Robert C. Post, Chief, Food Standards and Ingredients Branch Food Safety and Inspection Service (FSIS), USDA, Washington, DC 20250

Food Safety and Inspection Service (FSIS) is directly involved in the changes occurring in food ingredients use. The FSIS responds to petitions from industry, trade groups, academia, and other research organizations for the use of new ingredients and the new use of existing ingredients in meat and poultry products. The Agency confirms the safety and evaluates the technical efficacy of food ingredients intended for use in meat or poultry products. The FSIS maintains its own list of approved substances separate from that maintained by the Food and Drug Administration (FDA).

Several trends have spurred the creativity in ingredient use, viz., globalization, labeling reform, a "marketing quality" concept and industry and consumer concerns for safe, "healthy" ingredients, all of which translates to safe and healthy foods. The trend for safe and healthy foods is seen in the increased interest in the use of preservatives, particularly antimicrobial agents.

To date, only a limited number of antimicrobial substances have been approved for direct use in meat and poultry products. However, in this era of greater pathogen reduction efforts, there is a growing interest in antibacterial substances produced by certain strains of bacteria, e.g., nisin, a bacteriocin, for use in meat and poultry products. The use and application of these ingredients will require an integrated review by both FSIS and FDA.

#### INDUSTRY PERSPECTIVES ON THE USE OF NATURAL ANTIMICROBIALS AND INHIBITORS FOR FOOD APPLICATIONS

Graham W. Gould, formerly Unilever Research Laboratory, Colworth House, Sharnbrook, Bedfordshire, UK

The wide range of extremely effective naturally-occurring antimicrobial systems include animal-derived ones (e.g., enzymes such as lysozyme, lactoperoxidase; other proteins such as lactoferrin, lacto-ferricin, ovotransferrin, serum transferrins; small peptides such as histatins, magainins; the immune system), plant-derived ones (e.g., phytoalexins, low MW components of herbs and spices; phenolics such as oleuropein; essential oils) and ones derived from microorganisms (e.g., bacteriocins such as nisin, pediocin).

An increasing number of such natural systems is being deliberately utilized for food preservation, or being explored for such use. The future potential is substantial, particularly as the efficacy of these systems is demonstrated in additive or synergistic combinations with some of the other microorganism-inhibitory factors that we can employ to improve the safety and keepability of foods.

While "naturalness" per se is not necessarily a justifiable objective for these developments, the use of natural inhibitors as components of systems that can together enhance the effectiveness of preservation, with advantages in product quality and safety, does not arguably justify pursuit.

## THE QUALITY AND SAFETY OF AQUACULTURED FISHERY PRODUCTS SYMPOSIUM

#### INTRODUCTION TO AQUACULTURE

Roy E. Martin, Vice President, Science and Technology, National Fisheries Institute, 1525 Wilson Blvd., Arlington, VA 22209

An overview will be presented of the current status of Aquaculture in the U.S. with reference to worldwide developments. Aquaculture, as the fastest growing section of the agriculture, must begin taking its rightful place in our food economy.

#### CHEMICAL/PHYSIOLOGICAL PERSPECTIVES

Dr. Gunner Finne, Silliker Laboratories of Texas, 201 E. Holleman Drive, College Station, TX 77840

As compared to other muscle foods, fish and shellfish are characteristically high in low molecular weight organic nitrogen compounds. The concentration and composition of the non-protein nitrogen fraction are dependent on species, season, spawning migration, environmental conditions, diets, etc. In shrimp, this fraction is especially high in free amino acids which serve as osmoregulations during adaptation to changes in environmental salinity. When white shrimp, *Penaeus vannamei*, were held at different salinities, the free amino acid concentration was 81% higher at 50 as compared to 10 ppt. Flavor evaluation by trained sensory panel showed a significant flavor difference between shrimp held at 10 and 50 ppt, and 30 to 50 ppt, but not between 10 and 30 ppt. If changes in salinity are performed prior to harvesting, it is important to allow shrimp a long acclimation period in order to achieve maximum flavor enhancement at a maximum yield.

#### MICROBIOLOGICAL PERSPECTIVE — FIN-FISH

Dennis Westhoff, Ph.D, Chair, Department of Animal Sciences, University of Maryland, College Park, MD 20742

As part of a broader symposium, this paper will focus on the microbiological safety of aquacultured fin-fish. Intensive aquaculture of fin-fish in ponds or tanks may result in high bacterial loads on fish. Aquacultured fin-fish exposed to large populations of bacteria from environmental sources, or as a result of poor management and handling, could represent an increased risk of infection for both the fish and humans. The microflora of aquacultured fin-fish species is not well studied. Attention has been focused primarily on microorganisms that are pathogenic to the fish. Sources of microorganisms on the surface of fin-fish and the significance of indigenous and non-indigenous pathogens will be emphasized. An assessment of the probable risk posed by aquacultured fin-fish compared to wild fish will be made based on a review of microflora studies, consumption statistics and reported outbreaks and causes.

#### MICROBIOLOGICAL PERSPECTIVE — CRUSTACEANS

Ranzell Nickelson II, Ph.D., Vice President of Technical Services, Silliker Laboratories Group, Inc., 900 Maple Road, Homewood, IL 60430

Shrimp is an important and expensive commodity in international trade. The explosion in production of aquacultured shrimp has generated many interesting questions and controversies related to the quality and safety of this product. *Salmonella* has been used as an indication of poor sanitation in wild caught shrimp, but may, at times, be a part of the natural flora of pond-raised shrimp. Since large portions of pond-raised shrimp are cultured in tropical and sub-tropical climates, *Vibrio cholerae* becomes a public health concern. Changes in salinity, close confinement, water quality, use of sub-therapeutic antibiotics all complicate the microbiological quality and safety of aquacultured shrimp.

## **MICROBIAL SAFETY OF MOLLUSCAN SHELLFISH**

Gary E. Rodrick,\* Professor, and D. Dixon, University of Florida, Department of Food Science & Human Nutrition, Gainesville, FL 32608

Molluscan shellfish are well known for their ability to concentrate microorganisms from their seawater environment through filter feeding mechanisms. If harvested from polluted waters, temperature abused, eaten raw and/or improperly cooked, they can serve as a vector for transmitting viruses, bacteria and parasites that cause human disease. In addition, despite strict federal and state molluscan shellfish regulations, the consumption of raw and molluscan shellfish has caused significant disease and even death, to a highly identifiable group of "at-risk" consumers. This is primarily during warm water months in the Gulf of Mexico when high numbers of vibrios may occur in the shellfish harvesting waters. This presentation will briefly review the major microbial agents transmitted by the consumption of raw molluscan shellfish, innovative processing techniques to eliminate or reduce the internalized pathogens, and educational efforts to prevent these infections especially in the "at-risk" consumers.

## **RESIDUES IN AQUACULTURED PRODUCTS**

Dr. Inocencio Higuera-Ciapara\* and L. O. Noriega, CIAD, A.C., P.O. Box 1735, Hermosillo, Sonora, Mexico

Aquaculture has had to rely on the use of known therapeutic agents for the control of devastating diseases. However, if aquaculture products are to maintain a growing demand in the industrialized countries, they should be free from undesirable toxic residues. Recently, serious concern over the presence of hazardous bioactive compounds in edible tissues of farmed aquatic animals has arisen. Antibiotic residues which cause elicitation of resistance among pathogenic bacteria have played a major role in severe mass mortalities. Also, the presence of chemical contaminants originating in agricultural effluents or industrial discharges may lead to contamination of aquaculture products by pesticide residues, polychlorinated biphenyls, heavy metals (lead, cadmium, mercury) or petroleum hydrocarbons. It is of extreme importance that the aquaculture industry implement accurate and effective diagnosis and therapeutic programs which will not compromise the wholesomeness of the end-products.

## **QUALITY AND SAFETY OF VALUE-ADDED FRESHWATER FISH PRODUCTS**

Yao-wen Huang,\* Associate Professor, Department of Food Science and Technology, University of Georgia, Athens, GA 30602-7610, and Chung-yi Huang, Associate Professor, Department of Food Processing, National I-Lan Institute of Agriculture and Technology, I-Lan, Taiwan, ROC

The growing consumer demand for convenience foods as well as the demographic trends, including increases in two-career families and single parent households, have made precooked and ready-to-cook entrees very important in today's food industry. Value-added products made from freshwater species such as precooked or uncooked battered and breaded catfish fillet, marinated catfish, smoked rainbow trout and precooked crawfish are available in today's markets. While consumers are enjoying these products, the microbiological quality and potential safety issues need to be re-evaluated. For example, quality and safety of breaded products depend on the fish and batter, and smoked products depend on salt level, heat processing and packaging systems. The sanitary condition of workers and equipment are also important factors to affect both the quality and safety. Value-added freshwater fish products are becoming important in the average American's diet and are expected to increase in the future. The good manufacturing practice for these products need to be considered to assure quality and safety.

## **A SYMPOSIUM ON RISK MANAGEMENT (cont'd.)**

*Sponsored by the Grocery Manufacturers of America*

### **RISK MANAGEMENT: Control Practices and Their Impact**

#### **MANAGING RISKS FROM THE INDUSTRY PERSPECTIVE**

A. C. Baird-Parker, Unilever Research, Sharnbrook, Bedfordshire, UK

The paper will discuss the concepts and procedures applied by the food industry to assess and assure the microbiological safety of food. It will consider the evolution of such procedures over the past seventy years, and what further knowledge and understanding is required to achieve a more secure and science-based microbiological risk assessment procedure more widely applicable to industrially produced foods.

Amongst the discussed will be: ranking of foods according to their relative risks; testing and measurement of microbial numbers in foods for compliance with a microbiological criterion; design of a food process to achieve a particular performance standard e.g., destruction or inhibition of a particular number of microorganisms; and use of risk management procedures such as HACCP and HAZOP.

The paper will also consider some of the difficulties with applying formal and quantitative risk assessment procedures, such as those used to assess the toxicological risks associated with the use of a chemical substance.

#### **ECONOMIC IMPACT OF RISK ASSESSMENT/MANAGEMENT PRACTICES**

Jerome J. Kozak, MPH, Senior Vice President, International Dairy Foods Association, 1250 H. Street N.W., Suite 900, Washington, DC 20005

When one examines the efficacy of public health programs regarding food safety, ultimately the discussion must focus on the importance of building strong industry, consumer, regulatory, academia and health care partnerships. In many instances, programs are designed to address perceived risks as opposed to ones that are based on sound scientific principles. The dairy industry has had numerous examples of such problems involving risk concerns, such as *Listeria monocytogenes*, *Salmonella*, animal drug residue and more recently BST. It is important to define acceptable risk and best to use resources in addressing these concerns.

### **Education and Communication of Risks**

#### **EDUCATION AND THE PUBLIC'S UNDERSTANDING OF RISK—THE ROLE OF INDUSTRY, GOVERNMENT, AND ACADEMIA**

James M. Jay, University of Nevada Las Vegas, Las Vegas, NV 89154

By and large, consumers have a very poor understanding of microorganisms that are normally associated with fresh foods and the significance of such organisms relative to health risks. The three groups noted in the title should undertake some joint effort to, among other things, inform consumers that while microorganisms are present in all fresh/raw foods, health risks to most individuals are minimal. The population groups that are at greater risk need to be defined, and it is incumbent upon these individuals to take responsibility for avoiding high-risk foods. Since it is impractical to eliminate foodborne pathogens from all fresh foods without a terminal destructive process such as irradiation, the risks and benefits of consuming such foods need to be assessed by the various at-risk population groups.

### **DAIRY SYMPOSIUM II — SANITATION AND NEW APPROACHES TO BETTER DAIRY PRODUCTS**

#### **SURVIVAL AND INACTIVATION OF BIOFILM BACTERIA ON DAIRY AND MILKING EQUIPMENT SURFACES**

Amy C. Lee Wong, Ph.D., University of Wisconsin, Food Research Institute, 1925 Willow Drive, Madison, WI 53706

Biofilms have been recognized as a potential source of contamination in food processing environments which may lead to product spoilage and/or decrease transmission. Biofilm formation is a dynamic, multi-step process.

Initially, bacterial cells are transported to the surface and reversibly attach to it. Some of these cells become irreversibly adsorbed, grow and produce extracellular polymeric substances which have been shown to stabilize biofilms. Portions of the biofilm may detach and subsequently colonize other parts of the system. A concern with biofilm bacteria is that they may be more resistant to the effects of antimicrobials than their free-living (planktonic) counterparts. Hence, different strategies might be needed for their effective removal and/or inactivation. This presentation will describe various aspects of biofilm formation on dairy and milking equipment surfaces. The efficacies of detergents and sanitizers in removing and/or inactivating biofilm bacteria will be discussed.

#### **SHOULD MICROBIAL BIOFILMS BE A CONCERN IN THE DAIRY INDUSTRY**

Edmund A. Zottola, Ph.D., Department of Food Science and Nutrition, 1334 Eckles Avenue, University of Minnesota, St. Paul, MN 55018

Microbial biofilms are defined as colonies of microorganisms closely associated with a surface. Microbes are attracted to a surface by various forces, deposit and develop physical attachment to the surface. As these microbes grow and multiply, the newly formed cells attach to the surface, to each other and form a confluent growing colony of microbes. This complex matrix of bacterial cells and polysaccharide attachment material attracts and traps other microorganisms, debris and nutrients. This material serves as a food source for the organisms and growth can continue uninterrupted for a considerable amount of time. A mature biofilm will release cells into the environment and these cells can become a source of contamination if the biofilm is associated with a food contact surface. Control of microbial growth and disruption of biofilms on food contact surfaces is a major concern in the dairy food industry. Several researchers have suggested that the microbes in the biofilm are resistant to common sanitization practices. Others have shown that proper cleaning followed by sanitization will disrupt, destroy and control microbes in biofilms. This paper will discuss this research and focus on the control and prevention of microbial biofilms in the dairy food processing industry.

#### **FSEP — AGRICULTURE AND AGRI-FOOD CANADA APPROACH TO HACCP FOR FOOD PROCESSING**

Roger Wray, Agriculture and Agri-Food Canada, 110 Stone Road, West, Guelph, Ontario, Canada N1G 3W4

The Food Safety Enhancement Program (FSEP) is Agriculture and Agri-Food Canada's (AAFC) approach to encouraging the development and implementation of (HACCP -based) programs in agri-food establishments and shell egg grading stations.

Canada's FSEP approach is to give the agri-food industry the responsibility to develop their own program according to well defined, internationally recognized HACCP principles. AAFC's role is to establish these parameters in consultation with industry and to monitor the effectiveness of their HACCP programs.

The dairy industry is represented by the National Dairy Council (NDC) who are actively working in cooperation with AAFC to develop generic HACCP models to be used by industry. Both NDC and AAFC have jointly agreed to having a fully implemented HACCP program by 1996.

#### **USE AND IMPLEMENTATION OF BIOLUMINESCENCE FOR RAPID HYGIENE MONITORING IN DAIRY PLANTS**

Joseph Zindulis, Ph.D., BioTrace Inc., 666 Plainsboro, Suite 1116, Plainsboro, NJ 08536

Conventional methods for hygiene monitoring in dairies typically generate results 1 to 2 days after the milk has been processed. In contrast, use of bioluminescence from the adenosine triphosphate (ATP) assay detects in minutes the presence of microorganisms and milk residue. The data suggests the method has potential to provide a more meaningful index of cleanliness than microbial enumeration alone. Tests have repeatedly demonstrated a decrease in ATP on surface and in rinse waters following cleaning and sanitation. This data has also been used to devise specifications for routine hygiene monitoring. Implementation of routine monitoring with the ATP assay has been successful both within the quality control laboratory and among sanitation and production crews. The ATP assay provides a fast, simple method capable of integration in Hazard Analysis Critical Control

Points (HACCP) and enables production staff to proceed either directly into manufacturing or to take corrective action before start-up. The ATP method appears to have applicability for monitoring dairy hygiene at all production stages from the incoming tanker truck through packaging.

#### **IDENTIFICATION AND CONTROL OF PROCESSING VARIABLES THAT AFFECT THE QUALITY AND SAFETY OF DAIRY PRODUCTS**

Thomas Greutzmacher,\* Ph.D., Dean Food Company, 1126 Kilburn Avenue, Rockford, IL 61101, and Robert L. Bradley, Jr., Ph.D., University of Wisconsin, Madison, WI

The objective of this study was to increase quality and safety of fluid milk by eliminating post-pasteurization contamination as measured by extended shelf-life. Milk shelf-life was defined as the number of days for standard plate count to reach 20,000 CFU/ml in milk stored at 7°C.

Sequential analysis of the fluid milk processing system indicated the filling machine and pasteurizer are significant sources of post-pasteurization contamination. Aseptically sampled milk from the pasteurizer can be a source of contamination, especially after non-production days such as weekends. Inadequately cleaned or maintained equipment results in microbiological growth that contaminates production even with the use of sanitizers. There was no significant difference between shelf-life of milk sampled at the pasteurizer outlet and pasteurized milk storage tank outlet.

Shelf-life of milk in 236 ml containers was reduced 20 days compared with milk sampled prior to the filling machine. This indicated the filling machine was a significant source of contamination. Carton forming mandrels, filling heads and airborne microorganisms are sources of contamination within the filling machine. Milk shelf-life in cartons sterilized by cobalt 60 irradiation was not significantly different than in non-sterilized cartons when equipment was clean.

Eliminating sources of post-pasteurization contamination and proper cleaning followed by sanitizing with chlorine significantly increased milk shelf-life in paperboard containers to 20.4 days from an initial shelf-life of 9 days. Changing the sanitizing agent to peroxyacetic acid significantly increased milk shelf-life to 33.9 days. Enclosing the filling chamber and adding sterile laminar flowing air significantly improved microbiological quality of air inside the chamber and reduced variance of milk shelf-life samples.

#### **EUROPEAN FOOD PROCESSING EQUIPMENT HYGIENE STANDARDS SYMPOSIUM**

##### **INTRODUCTION**

Huib L. M. Lelieveld, Unilever Research Laboratory, Vlaardingen, The Netherlands

The design of a food processing plant may have a very significant effect on the microbiological stability and safety of the product. For a long time, food processors had no choice other than to design their methods of production and cleaning to cope with the available equipment. Often this resulted in high cleaning frequencies and severe cleaning procedures (long times at high temperatures, using high concentrations of alkaline and acid cleaning agents). Obviously much may be gained by using hygienic equipment. Health and spoilage problems may arise, however, due to confusion about the criteria for hygienic design. The contributions which follow will address this issue from various points of view.

##### **FOOD INDUSTRY PERSPECTIVE**

M. A. Mostert, Unilever Research Laboratory, Vlaardingen, The Netherlands

The design and performance of a process line often, if not usually, differs from design and performance expectations. Experience has taught us that one of the main reasons for this is the difference in interpretation of the terminology used and lack of understanding between the engineers of the equipment manufacturers and the microbiologists of the food manufacturers. Another important reason is that the food processor is not used to specify the engineering requirements. Regrettably, the equipment manufacturers, certainly in the case of standard equipment, do ask for such specifications. Also, manufacturers of equipment (from pipe connections to aseptic packing machines) usually refrain from mentioning the limitations (from a microbiological point of view) of their equipment.

It is therefore necessary that equipment manufacturers and food processors agree on the criteria of hygienic design and on the development and application of design standards and on methods to check — if needed — whether equipment meets the criteria agreed.

#### EQUIPMENT MANUFACTURER'S PERSPECTIVE

P. J. Skudder, APV Baker Ltd., Liquid Foods Division, Crawley, UK

It is absolutely vital that microbiological contamination of food is prevented during processing and filling operations. This can only be achieved through proper hygienic design of each individual component in the process line and of the complete processing system of which they form a part.

It is the responsibility of the equipment manufacturer to ensure that each component in the process line is designed to prevent microbiological contamination of the food and is capable of being satisfactorily cleaned in the manner specified. The manufacturer must also demonstrate that equipment has been practically validated to meet this vital criteria.

There is a need to agree on basic hygienic design criteria and the method of validation between equipment manufacturers, food processors and regulatory bodies. The approach by the European Hygienic Equipment Design Group seeks to achieve agreement between equipment manufacturers and food processors in order to recommend realistic and achievable standards to the regulatory bodies.

#### CEN AND EHEDG PERSPECTIVE

D. A. Timperly, Campden Food and Drink Research Association, Chipping Campden, UK

In June 1989, a European Communities Directive (89/392/EEC) relating to machinery was adopted. This states that machinery to prepare and process foodstuffs must be so designed and constructed as to avoid any risk of infection, sickness or contagion; some hygiene rules concerning the design and construction of machines are then given. From January 1, 1995 all food machinery in the EEC must comply with the requirements and bear the 'CE' (Conformité Européenne) symbol as evidence of compliance: without this, new machinery may not be sold in Europe. The CEN (Comité de Normalisation — the European Committee for Standardisation) was mandated to produce a 'horizontal' standard for all food machinery and 'vertical' standards for specific types of machinery. Many of these standards are under preparation and are intended to provide one means of conformity with the requirements of the Directive.

In November 1989, and independent consortium called the European Hygienic Equipment Design Group, was formed to develop guidelines and test methods for the safe and hygienic processing of food independent of any National or International standards. The group includes representatives from research institutes, the food industry, equipment manufacturers and government organization in Europe; formal links have also been established with the 3-A Sanitary Standards Organization in the U.S. There are currently eleven subgroups of the EHEDG carrying out research projects on various aspects of hygienic design.

The EHEDG was formed because it was considered that there was a need to:

- ensure that food products are processed hygienically and safely,
- provide standards to organizations with specialist views on hygienic aspects of equipment design (several members of EHEDG are members of CEN committees),
- ensure that in the future there will be no confusion as to whether and under which conditions equipment is microbiologically safe for the processing and packing of food,
- identify areas where knowledge of hygienic and aseptic design is insufficient and to encourage research and development in these areas.

#### THE GOVERNMENT PERSPECTIVE

B. Mitchell, Ministry of Agriculture, Fisheries & Food, London, UK

The U. K. Government's role in ensuring the Microbiological Safety of Food can perhaps best be summarized by the terms: legislation, investigation and education. Each will form a section of the paper discussing how they impinge on the hygienic design of equipment. Legislation will be discussed in terms of the Food Safety Act and the Food Hygiene Regulations, and

reference will be made to the BC Council Directive 93/43/EEC on the Hygiene foodstuffs. The investigation section will briefly describe research funded by the Ministry of Agriculture, Fisheries and Food on aspects of hygienic design, primarily at the Campden Food and Drink Research Association. The Education Section will refer to advice presented by the Richmond Committee and will outline the steps the Government has taken to influence hygienic design of equipment through bodies such as The British Standards Institute, the European Committee for Standardization (CEN) and the European Hygienic Equipment Design Group.

#### TEST METHODS AND THEIR DEVELOPMENT

J. T. Holah, Campden Food and Drink Research Association, Chipping Campden, UK

Equipment cleanability can be assessed in three stages. Firstly, sectional drawings can be examined, secondly, the equipment can be examined itself and thirdly, providing the first two stages were satisfactory, a practical cleanability test could be undertaken. For many examples of open equipment this may not be necessary but for closed equipment, it is impossible to visually assess the cleanability of internal surfaces.

A subgroup 'Test Methods' of the EHEDG has been formed to produce hygienic design guidelines that can be verified by standard test procedures. A variety of test procedures for a number of equipment requirements including cleanability, pasteurizability, sterilizability and aseptic capability have been published for comment. To further develop equipment cleanability test methods, a group of organizations in Europe (including EHEDG Test Methods Sub Group members) received funding from the EEC to undertake the project 'test method development for the practical assessment of food processing equipment cleanability.'

Both microbiological and non-microbiological (organic soil) methods will be developed to allow the assessment of: (a) the fundamental cleanability of a single example of an item food production equipment to demonstrate compliance with EEC Directives; (b) a suitable number of an item of food production equipment as part of a structured Quality Assurance system to allow food equipment manufacturers to assure product quality; and (c) a whole production line within a food factory. In addition to the requirements, the size and complexity of the item of food equipment will determine certain test method conditions. Small pieces of equipment could be assessed where the whole of the equipment food contact surface would be evaluated. Large and/or complicated pieces of equipment could be assessed over the whole of their food contact surfaces using organic soiling methods or, using the principles of HACCP, areas thought to be a hygienic design hazard could be specifically assessed by microbiological techniques.

#### THE 3-A VIEWPOINT ON EUROPEAN STANDARDS

T. M. Gilmore, Dairy and Food Industries Supply Association, Rockville, MD

The bedrock of 3-A success in the U.S. is threefold. Importantly the tripartite nature of the 3-A program i.e., three interest groups—equipment fabricators, equipment users (processors), regulators—are equally involved in preparing 3-A Standards. Secondly is the consensual process of the deliberations. The third pillar is the zero defect goal which leads to high standards for cleanability and product protection. This means the state of the art, most scientific standards are, for the U. S., the ultimate bedrock.

The 3-A program was started 50 years ago and was the only program of its type in the world. But now 3-A faces its two greatest challenges: harmonization, or achieving equivalent results with international hygienic standards writing organizations, and an expanded role beyond the dairy industry here and abroad. Standards must not be a basis for trade barriers but must be based on solid science thereby eliminating regional idiosyncrasies—the "not invented here" syndrome. The larger purpose in harmony is to foster a harmony of attitude and minds which will usher in and nurture an atmosphere of mutual trust and credibility. 3-A is uniquely positioned to lead Europe and the U.S. into a new age.

#### THE CHALLENGE — HARMONIZATION OF HYGIENIC DESIGN CRITERIA

R. R. Maller, Thomas J. Lipton Co., Englewood Cliffs, NJ

Over the past 13 years, I have worked with Sanitation, Food Safety and Hygienic Design in U.S. food producing companies. In addition, I have had



the opportunity to work with colleagues in Europe and Asia in the quest to realize Hygienic Design in food processing/packaging equipment and facilities. In all regions of the world, methods exist for testing and evaluating equipment and varying hygienic design criteria have been developed for both equipment and facilities (e.g., EHEDG Guidelines, 3-A Standards, IDF Recommendations).

Although everyone's final goal is to install equipment and construct facilities that are hygienic, because hygienic criteria vary and test methods may be different, there is confusion about basic requirements for acceptable equipment and facilities for food processing.

The challenge is to develop worldwide testing standards and basic hygienic criteria that can be used by operating food companies (both big and small), equipment manufacturers, building material suppliers, equipment and facility installation contractors, and regulatory agencies.

## **CURRENT FOOD AND HEALTH RELATED SAFETY ISSUES SYMPOSIUM**

### **THE IMPACT OF INTERNATIONAL FREE TRADE ON FOOD SAFETY STANDARDS**

Kathy Ting, Acting Director, Western Europe Inter-America Division, International Trade Policy, Foreign Agricultural Service, United States Department of Agriculture, Room 5506, 14th and Independence, S.W., Washington, DC 20250

The implementation of the North American Free Trade Agreement (NAFTA) in 1994 and continued global liberalization of trade under the General Agreement on Tariffs and Trade (GATT) will increase focus on the impacts of food safety standards on international trade. Global trade in agricultural products is estimated at \$220 billion annually. These agreements will bring new disciplines to the use of food safety standards as barriers to trade. While countries will be free to choose what they consider an appropriate level of protection or acceptable risk, they will need to demonstrate that required measures to achieve that level of protection are scientifically based. NAFTA and other trade agreements are also likely to lead to increased international dialogue on food safety issues.

### **INTERNATIONAL FOOD SAFETY AND QUALITY STANDARDS**

Cathy Carnevale, Acting Director, Center for Food Safety and Applied Nutrition, Food and Drug Administration, 200 C Street, S.W., Washington, DC 20204-0001

The North American Free Trade Agreement (NAFTA) and the General Agreement on Tariffs and Trade (GATT) Uruguay Round Provisions on Sanitary and Phytosanitary Measures and Technical Barriers to Trade (called Standards-Related Measures in GATT) will confer a new status on international food safety and quality standards. What are these so-called international standards, who sets them, and what is their basis? How are they dealt with under free trade agreements versus national standards? International and national standards, codes, guidelines, processing and production methods, including measures used to enforce country requirements, will be discussed in the context of free trade agreements in force.

### **DOES INTERNATIONAL FAIR TRADE MEAN COMPROMISED FOOD SAFETY STANDARDS? IMPACT ON SEAFOOD SAFETY**

Cameron Ray Hackney, Virginia Polytechnic Institute and State University, Department of Food Science and Technology, Blacksburg, VA 24061

The U.S. already imports considerable amounts of food products. While foodborne outbreaks have been traced to imported products, there is little reason to believe that fair trade agreements will compromise food safety. The USDA requires that meat processed in other countries follow similar processing procedures to those used in the U.S. Seafood will be used as the primary example of imported products, since over half of the seafood consumed in the U.S. is imported. The current cholera pandemic has not resulted in increased cholera outbreaks in the U.S. Just the reverse, more *Vibrio* outbreaks are associated with fresh domestic products. Certain viruses such as Hepatitis A may be of concern, but international travelers are far more likely to spread it, than imported foods. There is concern over certain pesticides and other contaminants that may be more prevalent in imported products. MOUs and proper inspection can do much to limit this concern.

### **POULTRY SAFETY AFTER NAFTA**

John A. Marcy, Assistant Professor, The Center of Excellence for Poultry Science, University of Arkansas, B114 Animal Science Building, Fayetteville, AK 72701

Before the signing of the North American Free Trade Agreement (NAFTA), rumors, press releases, editorials and even some debates and discussion abounded in an attempt to sway opinion, either popular or political, for or against the agreement. Some of these discussions dealt with food safety, principally from the standpoint of pesticides and the ominous "Circle of Death" concept ... U.S. exports of pesticides not approved for use in the U.S. to countries that export produce to the U.S. and the belief that these same banned pesticides will return to the U.S. on imported produce. Little discussion dealt with the free trade of animal products across North America. This paper will:

- detail differences in poultry production and processing across North America;
- discuss trade of poultry across borders and how that may change; and
- relate the above to concerns of food safety within the U. S.

### **HANTAVIRUS PULMONARY SYNDROME (HPS) — AN EMERGING PUBLIC HEALTH THREAT**

Richie Grinnell,\* R. S., M.P.H., and Rodger Gollub, M.D., United States Public Health Service, Indian Health Service, 505 Marquette N.W., Suite 1502, Albuquerque, NM 87102

In the spring and summer of 1993, a new illness was identified in the four state region of the southwest United States where New Mexico, Arizona, Colorado and Utah come together. Now known officially as the Hantavirus Pulmonary Syndrome (HPS), the illness is caused by infection with a newly discovered virus carried by rodents. Intense public health activities surrounding this new disease included recognition, surveillance, investigation, treatment, environmental cleanup, public policy and prevention. All of these steps required collaboration between the Federal Centers for Disease Control and Prevention, the Indian Health Services, the Navajo Tribal Health Department, four state health departments, public and private physicians, hospitals and laboratories. Most importantly, the Public Health response required close collaboration between the disciplines of Environmental Health Science, Health Education and Medicine. The history surrounding HPS may serve as a model of response to a new or emerging public health threat.

### **USE OF FOODBORNE DISEASE DATA FOR HACCP RISK ASSESSMENT: A NEW APPROACH IN THE STATE OF NEW YORK**

John J. Guzewish, R.S., M.P.H., Chief of Food Protection, New York State Department of Health, II University Place, Room 404, Albany, NY 12203-3313

Limitations in the way foodborne disease data are analyzed and reported make it difficult to use the data for Hazard Analysis Critical Control Point (HACCP) risk assessment. This warranted the creation of a new system of classification and analysis. Foodborne disease data from reported outbreaks in New York State between the years 1980-1991 (1528 outbreaks involving 31,675 cases) were reviewed to develop two new categories by which foodborne disease vehicles were classified: Methods of Preparation and Significant Ingredient. In addition, the current CDC list of contributing factors were expanded to more accurately reflect common problems encountered in these outbreaks. Data grouped by this method can be more readily used for the hazard analysis and identification of Critical Control Points; (CCP's) steps of the HACCP system. Two dimensional tables of this new data show trends in preparation methods, ingredients, and contributing factors that can be used for risk assessment of establishments and their menus. A more detailed table shows agents of concern and likely CCPs associated with specific ingredients for each method of preparation that more closely links foodborne disease data with HACCP. Increased support of foodborne disease surveillance would provide the data needed to make this new system a valuable tool for use in HACCP risk assessment.

# Federal Register

[Docket No. 94N-0243]

Certification for Exports; Revised  
Compliance Policy Guide; Availability

**Agency:** Food and Drug Administration, HHS.

**Action:** Notice.

**Summary:** The Food and Drug Administration (FDA) is announcing the availability of a revised Compliance Policy Guide (CPG) 7150.01 entitled, "Certification for Exports." Firms exporting products from the United States are often asked by foreign customers or foreign governments to supply a certification relating to products subject to the Federal Food, Drug, and Cosmetic Act and other acts that FDA administers. FDA has historically issued a number of different types of such certificates, e.g., Certificates of Free Sale, Certificates of Export, Certificates to Foreign Governments, and most recently the European Union Health Certificate for Fishery Products. With expanding world trade, ongoing international harmonization initiatives, and developing international agreements, pressures on FDA to issue more certificates for U.S. products are escalating. Therefore, FDA has revised the CPG to clarify that it is the responsibility of the certificate requestor to provide information that will allow FDA to issue a Certificate for Export and to provide guidance on the preparation of such certificates, including model forms. The revised guidance will improve agency uniformity and consistency in providing export certifications for FDA-regulated commodities.

**Addresses:** CPG 7150.01 may be ordered from the National Technical Information Service (NTIS), U.S. Department of Commerce, 5285 Port Royal Rd., Springfield, VA 22161. Orders must reference NTIS order number PB94-204401 and include payment of \$9 for each copy of the document plus \$2 shipping and handling. Payment may be made by check, money order,

charge card (American Express, Visa, or Mastercard), or billing arrangements made with NTIS. Charge card orders must include the charge card account number and expiration date. For telephone orders or further information on placing an order, call NTIS at (703) 487-4650. CPG 7150.01 is available for public examination in the Dockets Management Branch (HFA-305), Food and Drug Administration, rm. 1-23, 12420 Parklawn Dr., Rockville, MD 20857, between 9 a.m. and 4 p.m., Monday through Friday.

**For Further Information Contact:** Steven M. Solomon, Office of Regulatory Affairs (HFC-230), Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, 301-443-1500.

**Supplementary Information:** This revised CPG describes current agency views on certificates requested by U.S. firms to facilitate the exports of FDA-regulated products to other countries. The agency recognizes the current importance of fulfilling requests for export certificates, however, FDA's long-term policy is to work towards the reduction or elimination of export certificates by finding other means to satisfy other countries' need for reassurance about imported products. The new guide replaces CPG 7150.01 entitled "Certificates of Free Sale" that was issued in 1989.

The statements made in CPG 7150.01 are not intended to bind the courts, the public, or FDA or to create or confer any rights, privileges, immunities, or benefits on or for any private person, but are intended merely for internal FDA guidance.

Dated: August 15, 1994.

Ronald G. Chesemore,  
Associate Commissioner for Regulatory Affairs.

[FR Doc.94-22033 Filed 9-6-94; 8:45 am]

# 3-A Sanitary Standards

## Action Notice of 3-A Sanitary Standards

During the May 1994 meeting of the 3-A Sanitary Standards Committees there were 13 tentative documents approved for signature, publication and general distribution. Seven were amendments, two were new 3-A standards or practices while the remaining four were revisions.

The following amendments all have an effective date of August 20, 1994.

1. 09-09 A1 Amendments to 3-A Sanitary Standards for Sensors and Sensor Fittings and Connections Used on Milk and Milk Products Equipment.
2. 20-17 A2/20-17 A3 Amendments to 3-A Sanitary Standards for Multiple-Use Plastic Materials Used as Product Surfaces for Dairy Equipment.
3. 32-01 A1 Amendments to 3-A Sanitary Standards for Uninsulated Tanks for Milk and Milk Products.
4. 37-01 A1 Amendments to 3-A Sanitary Standards for Pressure and Level Sensing Devices.
5. 54-00 A2 Amendments to 3-A Sanitary Standards for Diaphragm Type Valves for Milk and Milk Products.
6. 605-04 A1 Editorial Correction to 3-A Accepted Practices for Permanently Installed Sanitary Product Pipelines and Cleaning Systems Used in Milk and Milk Processing Plants.

The following revised or new 3-A documents have an effective date of November 20, 1994.

1. 33-01 Revisions to 3-A Sanitary Standards for Polished Metal Tubing for Dairy Products.
2. 61-01 Revisions to 3-A Sanitary Standards for Steam Injection Heaters for Milk and Milk Products.
3. 65-00 New 3-A Sanitary Standards for Sight and/or Light Windows and Sight Indicators in Contact with Milk and Milk Products.
4. 604-04 Revisions to 3-A Accepted Practices for Supply Air Under Pressure in Contact with Milk, Milk Products and Product Contact Surfaces.
5. 609-01 Revisions to 3-A Accepted Practices for a Method of Producing Steam of Culinary Quality.
6. 611-00 New 3-A Accepted Practices for Farm Milk Cooling and Storage Systems.

### ERRATA

1. The 3-A Sanitary Standards for Multiple Use Plastic Materials Used as Product Contact Surfaces for Dairy Equipment, Number 20-17 were amended to include a new generic class of plastic material, effective September 1993. This serial number is corrected from 20-18 to 20-17, Amendment 1.
2. Standards for Uninsulated Tanks for Milk and Milk Products, Number 37-01 is corrected from 32-02 to 32-01, Amendment 1.
3. The amendment to the 3-A Sanitary Standards for Diaphragm-Type Valves for Milk and Milk Products, Number 54-00, Amendment 1 is corrected to 3-A Sanitary Standards for Diaphragm-Type Valves for Milk and Milk Products, Number 54-00, Amendment 2 (formally 08-17B).
4. The amendments to Part One of the 3-A Sanitary Standards for Sensors and Sensor Fittings and Connections Used on Milk and Milk Products Equipment, Number 09-09 is corrected by deleting the second "Number 09-09" and adding "Amendment 1."

The new, amended or revised standards will be available from IAMFES or the 3-A Secretary 30 days before their effective dates.

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1245 Reamwood Ave. Sunnyvale, CA 94089

**Tim DonLevy**  
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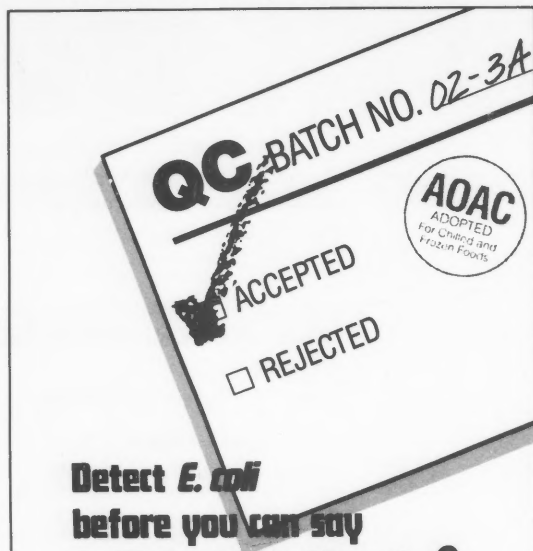
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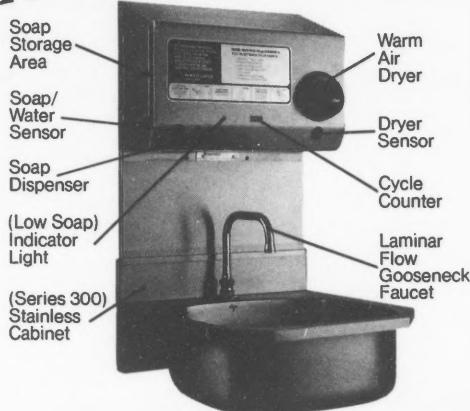
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# Industry Products

## Oxine<sup>®(FP)</sup> for Biofilm Formation

**B**CI, Incorporated is pleased to offer Oxine<sup>®(FP)</sup>, a unique USDA D2 sanitizer, for use in a full range of food processing applications. The chlorine dioxide chemistry in Oxine<sup>®(FP)</sup> is effective against a wide range of microorganisms including *Listeria monocytogenes*.

Oxine<sup>®(FP)</sup> has been evaluated directly against other "new generation" sanitizers and was found to be significantly better. Its activity against biofilms makes it ideal for use in cooling and chillwater systems, CIP sanitation and process water. In addition, Oxine<sup>®(FP)</sup> may be fogged in overhead areas, ducts, etc. No toxic by-products are produced as with chlorine, and Oxine<sup>®(FP)</sup> has low odor and low corrosive potential.

## Reflectoquant Analysis System Available for Contamination Monitoring

**E**M Science has developed the Reflectoquant System for contamination monitoring and analysis. The technologically advanced system features the new RQflex Meter, a handheld instrument for use with Reflectoquant Test Strips.

The Reflectoquant System enables the user to obtain test results on the parts-per-million level using the test strip read by the meter, with results available in 60 s. The RQflex Meter reads test strips in 30 different analytes, including chromium, nitrate and peroxide. The instrument features programming by the bar code method and results storage.

The Reflectoquant System features applications for a broad spectrum of industries and disciplines, including industrial and environmental laboratories, environmental field screening and waste water management, the food and beverage industry, electroplates, and other industries requiring process control and environmental monitoring.

## Breakthrough in Summer and Winter Ventilation

**A**t the Plant Engineering show in Chicago, TechMaster Inc. introduced the SeasonMaster Air Direction Controller that allows the air flow direction from the EnergyMaster make-up air ducts to be easily changed between the cold weather upward position and the warm weather downward position. This is accomplished with a simple adjustment on the unit that takes only seconds and is easily made from the floor level; rather than manually rotating the duct 180° at a high labor cost.

During the year, especially in between the seasons, daily temperatures often fluctuate up and down. In order to prevent the work area from overheating or over-cooling, this adjustment should be made whenever necessary, day to day, or even shift to shift.

filter is suitable for a wide range of applications in food and pharmaceutical processing where solids from one micron to one-half inch are removed at feed pressures to 100 psi. Applications involve sweeteners, beverages, brines, dairy products, cooking oils, personal care products and biochemicals.

The Sanitary LP Series filter's housing is a single piece, spun metal unit for easier cleaning. All surfaces are stainless steel and electro-polished. Product contact area is 150 grit minimum. All internal seams are gas purge welded or back welded to eliminate voids and crevices where bacteria can hide and grow.

Cleanable, reusable filter bags are available in a variety of materials and mesh sizes. Bags include handles on flange and allows press fit placement for fast changeout. Built-in drain port makes bag removal for cleaning or changeout a cleaner, an easier task.

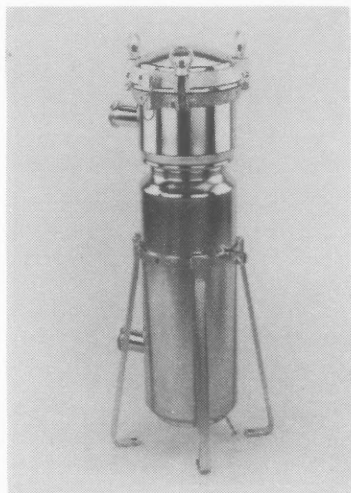
## New Indicate Acid

**W**est Agro, Inc., a manufacturer of cleaners and sanitizers for dairy and food processing facilities, announces a new product in its chemical sanitation line.

The "Indicate Acid" is a blended, non-foaming acid detergent with a unique soil indicating system used for soak and C.I.P. cleaning of stainless steel parts and equipment. As an acid cleaner it effectively removes mineral scale and carbon deposits. If the alkali cleaning step prior to acid cleaning fails in any way, the unique indicating dye will highlight any residual protein soils for easy inspection and correction.

## Nelson Jameson Introduces NoFoam/UF<sup>™</sup> Powdered Antifoam

**A** significant technical advancement, this product offers dairy processors a silicone-based antifoam which is compatible with ultrafiltration (UF) systems and does not permanently foul or harm membranes. This product,



## Low Cost Bag Filters for Solids Removal From Liquids in Food and Pharmaceutical Processing

**A** new, low cost liquid filter that meets 3-A standards for milk and milk product filters is now available from R-P Products. By meeting 3-A Standards, the Sanitary LP Series bag

with its "membrane-friendly" technology, complies with applicable FDA, EPA and USDA requirements.

NoFoam/UF Powdered Antifoam can be used as a process defoamer/antifoam in many dairy applications. Because NoFoam/UF does not permanently foul ultrafiltration membranes, it is ideal for use in whey. This is of particular value to producers of whey which is destined for further processing by ultrafiltration. Other uses include applications or processes such as in starter tanks, balance tanks or drying operations in which foaming presents a problem to the dairy processor.

FDA regulation 173.340 allows the use of silicone-based defoamer/antifoam agents as processing aids up to 10 parts active silicone per million parts foamer in all food products unless restricted by standards of identity.

## Paradigm 2000 Redefines Dairy Plant Sanitation

The Klenzade Paradigm™ 2000 products help lower the pH of cleaning solutions and wastewater, have the potential for reducing rinse water volumes and related cleaning cycle times, and also help enhance worker safety, based on the product line's unique chemistry. Klenzade developed this unique chemistry to address the needs expressed by the Dairy Industry. The patent pending Paradigm 2000 chemistry uses a proteolytic enzyme to replace chlorine, a surfactant/alkaline builder system to replace caustic, and an organic chelating agent to replace phosphates, for water conditioning, to prevent mineral film redeposition and minimize calcium phosphate formation. A sanitation program using Paradigm 2000 products in conjunction with an acid or pre-acid sanitizer greatly reduces corrosion potential, helping to minimize surface corrosion which can lead to microbial biofilm development.

## Weber Scientific's Micro Liquid Cleaner - An Effective and Economical Cleaner for Food & Dairy Labs

Micro® has proven to be effective in cleaning laboratory glassware and stainless steel, yet is mild, biodegradable, 100% rinseable and has no toxic/inhibitory characteristics.

A 2% solution of the highly concentrated Micro will completely remove all grease, food and protein residue from lab glassware when left to soak overnight. Pipets can be kept clean if placed into a 2% Micro solution immediately after use, followed by siphon rinse.

A chemical analysis for residue of Micro, with a 10 ppm limit of detection, found no Micro residue.

When tested in accordance with the Inhibitory Residue Test, Standard Methods for the Examination of Water and Wastewater, Micro exhibited no toxicity or inhibitory characteristics.



## New Automatic Hand Dryer

Electric-Aire, a division of World Dryer, has introduced a new automatic hand dryer that provides quality hand drying at a very low price.

The new dryer features touchless, automatic activation for improved sanitation and high air velocity for quick and efficient hand drying. It has a durable high-impact cover that is double insulated.

Designed to meet the needs of the janitorial supply market, the new automatic complements the line of stainless steel Electric-Aire hand dryers to create a complete assortment of products.

## Nutrient Products for Bioremediation

A new line of nutrient products designed for microbial stimulation during bioremediation has been introduced by Bioscience, Inc.

The products include MICROCAT-NPN, with a balanced ratio of nitrogen and phosphorus for optimum microbial stimulation; MICROCAT-N, providing high concentrations of nitrogen only and MICROCAT-P, with high concentration of phosphorus only.

The nutrients are sold as dry, free-flowing granules, in 50 lb. and 20 lb. containers. Other bioremediation products include vitamins, micronutrients and biostimulators for use with either naturally occurring microorganisms or microbial additives.

## Sterilex Has New Development

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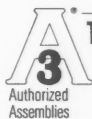
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B. Paid and/or Requested Circulation		
1. Sales through dealers and carriers, street vendors and counter sales	0	0
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# Coming Events

## 1994

### NOVEMBER

- 1, **Associated Illinois Milk, Food and Environmental Sanitarians Fall Annual Meeting**, at the Carlisle in Lombard, IL. For more information, contact Bob Crombie at (815) 726-1683.
- 1-2, **Food Plant Sanitation**, Toronto, Ontario, Canada; a course offered by the American Institute of Baking. For more information, call (800) 633-5137 or (913) 537-4750.
- 2-3, **North Dakota Environmental Health Assn. Annual Educational Conference** will be held at the International Inn, Williston, ND. For more information, contact Deb Larson at (701) 221-6147.
- 2-7, **Fifth Panamerican Dairy Congress, the International Fair of the Dairy Industry and Dairy Cattle Exhibition**, co-sponsored by the Panamerican Dairy Federation, FEPALA and the COLANTA Dairy Cooperative, will be held in Medellín, Columbia, South America.
- 3-4, **Bread and Rolls Production**, San Jose, CA; a course offered by the American Institute of Baking. For more information, call (800) 633-5137 or (913) 537-4750.
- 3-4, **Safety for the Supervisor**, Louisville, KY; a course offered by the American Institute of Baking. For more information, call (800) 633-5137 or (913) 537-4750.
- 7-9, **SERVS SAFE® Serving Safe Food Seminar**, in Boston, MA. Co-sponsored by the Massachusetts Restaurant Association, held at the Cambridge Howard Johnson. For additional information or to register, contact The Educational Foundation's customer service department at (800) 765-2122.
- 7-9, **SERVS SAFE® Serving Safe Food Seminar**, in New Orleans, LA. Co-sponsored by the Louisiana Restaurant Association, held at the Westin Canal Place. For additional information or to register, contact The Educational Foundation's customer service department at (800) 765-2122.
- 7-9, **SERVS SAFE® Serving Safe Food Seminar**, in San Francisco, CA. Co-sponsored by the California Restaurant Association, held at the San Francisco Marriott-Fisherman's Wharf. For additional information or to register, contact The Educational Foundation's customer service department at (800) 765-2122.
- 7-10, **Cookie and Cracker Technology for Allied and Associated Personnel**, Manhattan, KS; a course offered by the American Institute of Baking. For more information, call (800) 633-5137 or (913) 537-4750.
- 7-10, **Second Saudi Symposium and Exhibition on Food and Nutrition** will be held at King Saud University campus in Riyadh, Saudi Arabia. For more information, contact the Food Science Department at (966) 467-8407; FAX (966) 467-8394.
- 9-10, **Separations Technologies: Markets and Applications**. Location: Royal Sonesta Hotel, Cambridge, MA. For more information, contact Program Division, Technomic Publishing Company, Inc., 851 New Holland, Ave., Box 3535, Lancaster, PA 17604; phone (717) 291-5609; FAX (717) 295-4538. Toll-free U.S. and Canada: (800) 223-9936.
- 9-11, **Northeast Dairy Practices Council 25th Annual Meeting**, North Syracuse, NY. For more information, call NDPC at (315) 449-7547.
- 14-18, **Maintenance and Management**, Manhattan, KS; a course offered by the American Institute of Baking. For more information, call (800) 633-5137 or (913) 537-4750.

•17-18, **ISO 9000 Registration Workshop**, Manhattan, KS; a course offered by the American Institute of Baking. For more information, call (800) 633-5137 or (913) 537-4750.

•30-Dec. 2, **Muffin and Doughnut Technology**; Manhattan, KS; a course offered by the American Institute of Baking. For more information, call (800) 633-5137 or (913) 537-4750.

### DECEMBER

- 5-7, **SERVS SAFE® Serving Safe Food Seminar**, in Chicago, IL. Co-sponsored by the Illinois Restaurant Association, held at the Midland Hotel. For additional information or to register, contact The Educational Foundation's customer service department at (800) 765-2122.
- 5-7, **Food Ingredient Technology**, East Brunswick, NJ, a course offered by The Center for Professional Advancement. For more information, call (908) 613-4500.
- 5-7 **Good Manufacturing Practice (GMP) for the Food Industry**, East Brunswick, NJ; a course offered by The Center for Professional Advancement. For more information, call (908) 613-4500.
- 5-8, **Toxics Release Inventory Data Use Conference 1994: Building TRI and Pollution Prevention Partnerships**, The Park Plaza Hotel, Boston, MA. For more information, contact Madsen Marketing Strategies, 31 Kidder Avenue, Somerville, MA 02144; phone (617) 666-1431; FAX (617) 628-9297.
- 6-7, **Understanding Industrial Motor Controls**, Chicago, IL; a course offered by the American Institute of Baking. For more information, call (800) 633-5137 or (913) 537-4750.
- 6-7, **Hazard Analysis Critical Control Points (HACCP) — A Basic Concept for Food Protection...**, to be held at the University of California, Davis, CA. For more information, call (800) 752-0881 in California; (916) 757-8777 outside of California; or FAX (916) 757-8558.
- 7-9, **Basic Safety School**, Phoenix, AZ; a course offered by the American Institute of Baking. For more information, call (800) 633-5137 or (913) 537-4750.
- 12-13, **Thermal Processing of Foods I; Operation of Pasteurizer Equipment**. Fee to be established. For more information, contact Mr. A. W. Hydamak at (204) 474-9621; FAX (204) 261-1488.
- 13-14, **Understanding Industrial Motor Controls**, Kansas City, MO; a course offered by the American Institute of Baking. For more information, call (800) 633-5137 or (913) 537-4750.
- 14-15, **Farm Personnel Management Workshop**, LaCrosse, WI, offered by extension services of Iowa State University, University of Illinois, University of Minnesota and University of Wisconsin. For more information, call (608) 263-3485.
- 14-15, **Thermal Processing of Foods II: Testing of Pasteurizer Equipment and Controls**. Fee to be established. For more information, contact Mr. A. W. Hydamak, at (204) 474-9621; FAX (204) 261-1488.
- 28-Jan. 1, **National Milk Producers Federation Annual Meeting**, Dallas, TX; For more information, call National Milk Producers Federation at (703) 243-6111.

# 1995

## JANUARY

**•3-5, Milling for Cereal Chemists**, sponsored by the American Association of Cereal Chemists, will be held in Kansas State University, Manhattan, KS. For more information, contact Marie McHenry, AACC Short Course Coordinator, 3340 Pilot Knob Road, St. Paul, MN 55121. Phone (612) 454-7250; FAX (612) 454-0766.

**•9-Feb. 10, Dairy Technology Module II — Technology of Cheese and Concentrated Milk Products**. The principles and practices relating to the manufacture of cheese. Includes selection and evaluation of raw materials plus lactic cultures, processing, packaging, storage and distribution. Aspects of quality control, product testing, judging and grading associated with cheese production. Cost: \$873.00 For more information, contact Mr. A. W. Hydamaka, at (204) 474-9621; FAX (204) 261-1488.

**•10-12, Introduction to Food Chemistry**, sponsored by the American Association of Cereal Chemists will be held in Los Angeles, CA. For more information, contact Marie McHenry, AACC Short Course Coordinator, 3340 Pilot Knob Road, St. Paul, MN 55121; phone (612) 454-7250; FAX (612) 454-0766.

**•16-17, Wheat Gluten: Chemistry and Technology**, sponsored by the American Association of Cereal Chemists, will be held in Kansas City, MO. For more information, contact Marie McHenry, AACC Short Course Coordinator, 3340 Pilot Knob Road, St. Paul, MN 55121; phone (612) 454-7250; FAX (612) 454-0766.

**•18, Dough Modifiers**, sponsored by the American Association of Cereal Chemists, will be held in Kansas City, MO. For more information, contact Marie McHenry, AACC Short Course Coordinator, 3340 Pilot Knob Road, St. Paul, MN 55121; phone (612) 454-7250; FAX (612) 454-0766.

**•18-21, U.S. Dairy Forum**, sponsored by the International Dairy Foods Association, will be held at La Quinta Resort and Club in Palm Springs, CA. For more information, call (202) 737-IDFA.

**•19, Food Surfactants**, sponsored by the American Association of Cereal Chemists, will be held in Kansas City, MO. For more information, contact Marie McHenry, AACC Short Course Coordinator, 3340 Pilot Knob Road, St. Paul, MN 55121; phone (612) 454-7250; FAX (612) 454-0766.

## FEBRUARY

**•6-9, Freezing Technology Short Course**, on the UC-Davis Campus. This intensive course teaches the fundamentals of freezing specific commodities and includes hands-on demonstrations. To enroll or request more information, call toll-free in California (800) 752-0881. Outside of California, call (916) 757-8777.

**•8-10, Eighth Australian Food Microbiology Conference** to be held in Melbourne. Utilizing a mixture of local and international speakers, drawn from the key areas of the industry, Academia and Research, the aim of this conference is to provide a wide range of topics of interest to the Food Microbiology Industry. In addition, a poster session will be conducted. For more information, contact Kim King, Conference Secretariat, Food Micro '95, GPO Box 128, Sydney NSW 2001, Australia; phone 61-2-262-2277; FAX 61-2-262-2323.

**•13-14, 4th Annual Cheese Symposium to Introduce Product Research Results**, to be held in Burlingame, CA. The conference

focuses on the latest developments in cheese science and technology, and introduces the results of dairy products related research. To enroll or request more information, call toll-free in California (800) 752-0881. Outside of California, call (916) 757-8777.

## MARCH

**•2-4, Introduction to Statistical Methods for Sensory Evaluation of Foods**; a course to be offered at the UC-Davis campus. The fee is \$575.00 and includes one dinner, two lunches and the course text or manual. For more information or to enroll, call toll-free in California (800) 752-0881. Outside California, call (916) 757-8777.

**•6-8, Principles of Cereal Science**, a short course sponsored by American Association of Cereal Chemists will be held in Los Angeles, CA. For more information, contact Marie McHenry, AACC Short Course Coordinator, 3340 Pilot Knob Road, St. Paul, MN 55121; phone (612) 454-7250; FAX (612) 454-0766.

**•6-8, Sensory Evaluation: Overview and Update**, an additional course offered at the UC-Davis campus. The fee is \$575.00, or \$1,000 to attend both this and the "Introduction to Statistical Methods for Sensory Evaluation of Foods." For more information or to enroll, call toll-free in California (800) 752-0881. Outside California, call (916) 757-8777.

**•9-10, Fats, Oils and Substitutes in Baked Products**, a short course sponsored by American Association of Cereal Chemists will be held in Chicago, IL. Contact Marie McHenry, AACC Short Course Coordinator, 3340 Pilot Knob Road, St. Paul, MN 55121; phone (612) 454-7250; FAX (612) 454-0766.

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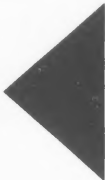
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## IAMFES

International Association of Milk, Food and Environmental Sanitarians Inc.

DFES  
10/94

Reader requests for information are sent to the appropriate company. Follow-up on reader requests are the responsibility of the company advertising.

The Advertisements included herein are not necessarily endorsed by the International Association of Milk, Food and Environmental Sanitarians, Inc.

Name \_\_\_\_\_ Title \_\_\_\_\_  
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101	114	127	140	153	166	179	192	205	218	231	244	257	270	283	296	309	322	335	348
102	115	128	141	154	167	180	193	206	219	232	245	258	271	284	297	310	323	336	349
103	116	129	142	155	168	181	194	207	220	233	246	259	272	285	298	311	324	337	350
104	117	130	143	156	169	182	195	208	221	234	247	260	273	286	299	312	325	338	351
105	118	131	144	157	170	183	196	209	222	235	248	261	274	287	300	313	326	339	352
106	119	132	145	158	171	184	197	210	223	236	249	262	275	288	301	314	327	340	353
107	120	133	146	159	172	185	198	211	224	237	250	263	276	289	302	315	328	341	354
108	121	134	147	160	173	186	199	212	225	238	251	264	277	290	303	316	329	342	355
109	122	135	148	161	174	187	200	213	226	239	252	265	278	291	304	317	330	343	356
110	123	136	149	162	175	188	201	214	227	240	253	266	279	292	305	318	331	344	357
111	124	137	150	163	176	189	202	215	228	241	254	267	280	293	306	319	332	345	358
112	125	138	151	164	177	190	203	216	229	242	255	268	281	294	307	320	333	346	359
113	126	139	152	165	178	191	204	217	230	243	256	269	282	295	308	321	334	347	360

This second Reader Service Card is provided to allow co-workers to also respond to companies of interest.

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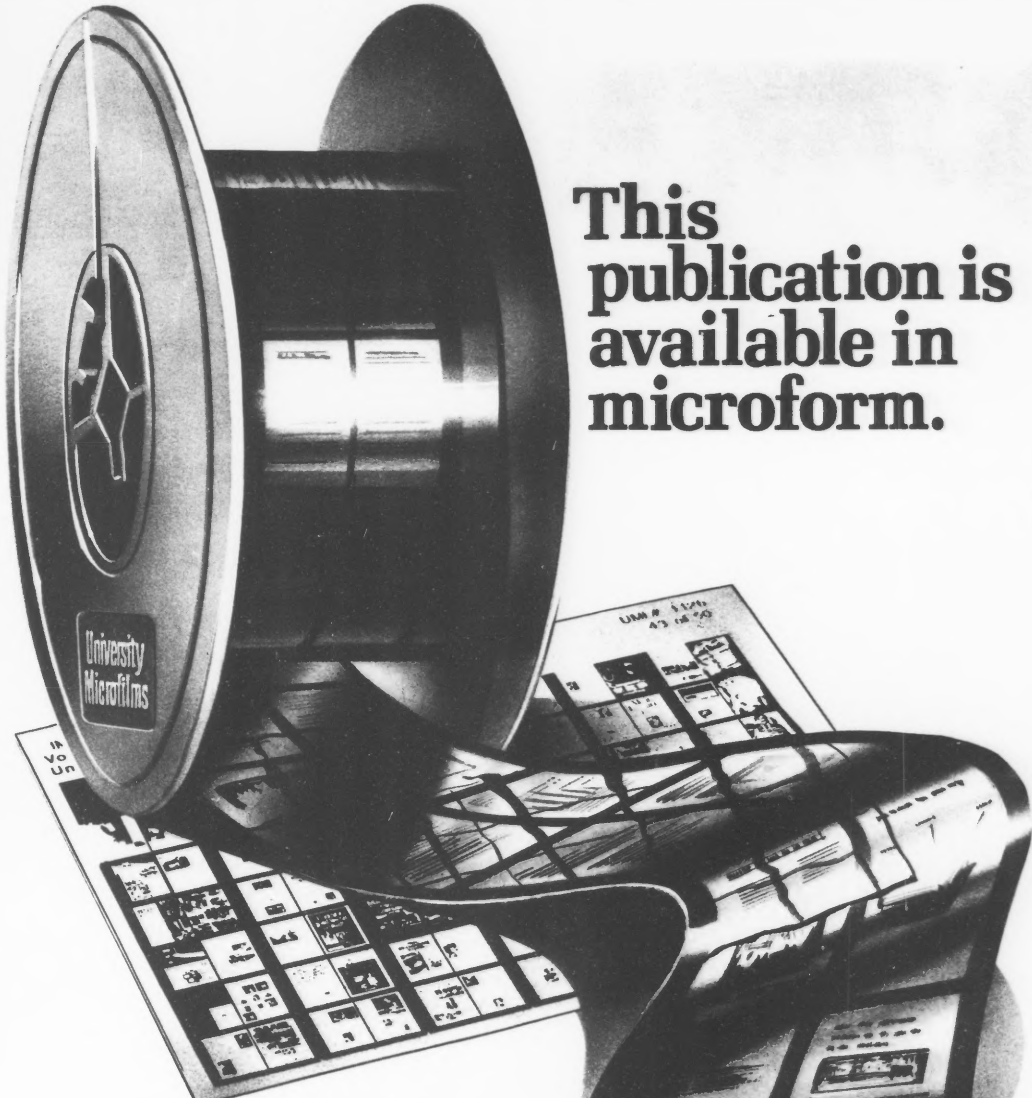
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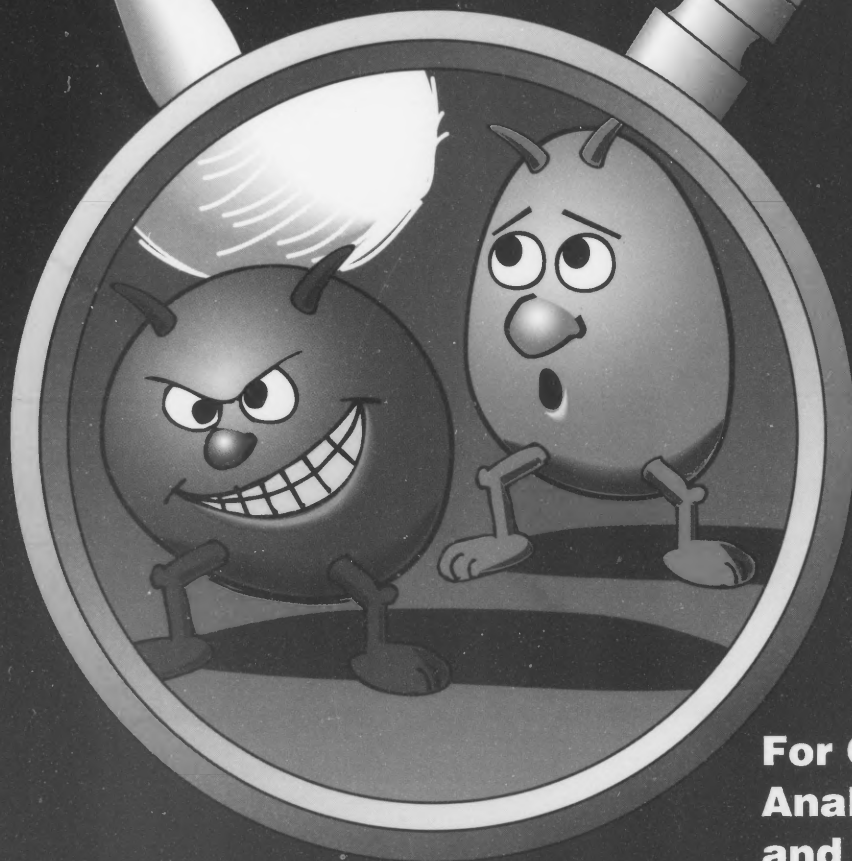
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