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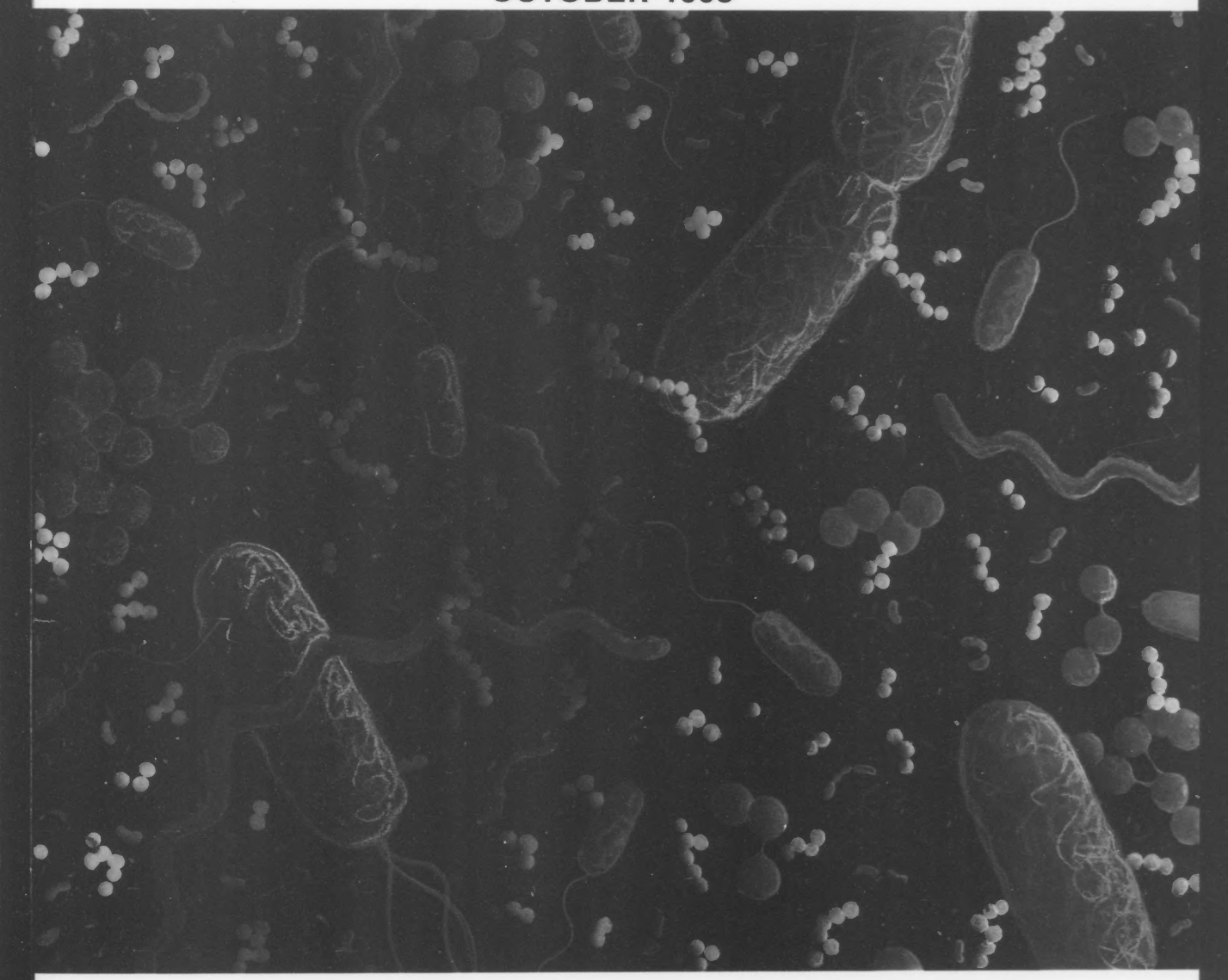
DAIRY, FOOD AND ENVIRONMENTAL

SANITATION

OCTOBER 1993



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IAMFES

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 Award 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 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.

Awards: First Place: $500 and an Award 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.

Ten finalists will be selected for the Poster Competition. The presentation must be mounted on a 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.

Award: First Place: $500 and an Award 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: Both a short abstract and an extended abstract must be submitted to the IAMFES office no later than December 15, 1993. No forms will be sent to entrants. Enclose two self-addressed, stamped postcards with your submitted abstracts.**

1. An original short abstract of the paper must be submitted on the blue abstract form from the September issue of IAMFES' journals. Indicate on the short abstract form whether the presentation is submitted for the Oral or Poster Competition.
2. One original and four copies of an extended abstract MUST BE SUBMITTED with the short abstract. Instructions for preparing the extended abstract follow. Attach one copy of the short abstract to each copy of the extended abstract and submit together with the original short abstract.
3. The presentation and the student must be recommended and approved for the Competition by the Major Professor or Department Head, who must sign both the short and the extended abstracts.
4. The work must represent original research done by the student and must be presented by the student.
5. Each student may enter only one (1) paper in either the Oral or Poster Competition.
6. All students will receive confirmation of acceptance of their presentations along with guidelines for preparing their Oral or Poster Presentations.
7. **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.***
8. Winners are announced at the Annual Awards Banquet. The ten finalists for the Oral Competition and the Poster Competition will receive complimentary tickets and are expected to be present at the Banquet.

Thoughts from the President

By
Harold Bengsch
IAMFES President

The Black Pearl Award

"THE ELUSIVE BLACK PEARL, SOUGHT AFTER FROM OCEANIA TO THE ORIENT BY EUROPEAN LORDS AND ASIAN EMPERORS ALIKE. ITS RARITY A SIGN OF DETERMINATION. ITS LUSTER A SIGN OF QUALITY. ITS ACQUISITION A SIGN OF EXCELLENCE."

So goes the ancient writing regarding the mystical "Black Pearl."

Thanks to the benevolence of Mr. Wilbur Feagan and the F&H Food Equipment Company, the IAMFES Board has created an additional recognition award category known as the "Black Pearl" Award. This award will be directed toward recognizing corporate commitment to food safety efforts. As such,

The Black Pearl Award recognizes a company for its outstanding achievement in corporate excellence in food safety and quality.

Qualifications Required for Nomination for the Black Pearl Award

- Be a corporate entity currently involved in/with the food industry.
- Not a previous recipient of the Black Pearl Award.
- Must have employees with active membership with IAMFES.
- Nominations *must* be submitted on IAMFES Nominating Award form. Award to be given to a company with all facilities included. However, a company may be a division of a larger corporate structure.

Criteria for Evaluating Nominations

- Contributions to public health principals and food safety.
- Food safety education activities.
- Evidence of support for the goals and objectives of IAMFES.
- Evidence showing promotion of ethical and fair business practices including employee relations, consumer relations, organizational operations and competitive interactions.
- Evidence of community/consumer relations to promote food safety.
- Employee programs to promote food safety.
- Products and/or services demonstrating a commitment to food safety.
- Evidence that facilities are designed with food safety and sanitation as a primary concern.
- Evidence of adherence to food safety regulatory requirements.
- Statement by corporate officer of commitment to principals of food safety and sanitation (not to exceed 250 words).

Nominating Form

- Basic information *must* be submitted on IAMFES nomination form.
- Three letters of support from IAMFES members.
- Supporting materials to include information on:
  - Food safety education activities involving employees, consumers and/or community efforts.
  - Approaches to employee training.
  - Approaches to assurance of product safety and/or quality research.
  - Any contributions to food safety and/or quality research.
  - Supporting materials demonstrating contribution to the goals and objectives of the IAMFES.
  - Regulatory compliance activity.
  - Ethical standards in competitive interactions.

It is our plan to present the first "Black Pearl" award at the 1994 annual IAMFES meeting in San Antonio, Texas. More will be said about this award in further issues of Dairy, Food and Environmental Sanitation.

Until next month!

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ABOUT THE COVER . . . *Photo courtesy of Promega Corp. , maker of the Enliten™ Rapid DiagnosticTests for food and dairy products. For more information contact Promega Corp., 2800 Woods Hollow Road, Madison, WI 53711-5399, (800) 356-9526 or 608-274-4330.*

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"Instructions to Contributors" can be obtained from the editor.

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On My Mind . . .

By
Steven K. Halstead, CAE
Executive Manager

is Floods — Part III . . .

By the time we returned to Des Moines from the Annual Meeting in Atlanta, most of the flood water had receded. For our colleagues downstream, this simply meant that their problems were just beginning. For us it meant cleanup. Massive amounts of cleanup.

Running water had been restored in Des Moines before we left for Atlanta and while we were there, it was declared safe to drink. Achieving that was a very difficult task.

The three main water pumps had been submerged when the Raccoon river went over the levee protecting the waterworks facility. As soon as they were above water again, they were picked up by huge military helicopters and taken to a repair facility. There they were disassembled and every part hand cleaned and sanitized. The problem was made worse by the fact that there was no running water to do all this.

By working around the clock, the pumps and motors were cleaned in about a week. In the meantime, the rest of the waterworks was cleaned and sanitized. The filters and filtration beds along with all the other equipment had to be disassembled, cleaned, sanitized and then reassembled. Once that was done, the water system could be "charged" and the cleaning of the hundreds of miles of water pipes could begin.

That process was accomplished with the use of chemical sanitizers and by simply flushing the system. You will recall that raw flood water had entered the system, so the whole thing was contaminated.

By this time, it was possible to isolate various sections of the system and to concentrate on bringing them on line one at a time. Each section was tested by randomly selecting water sampling sites and checking for contaminates and coliforms. The number of samples tested was large enough to insure adequate coverage. If all samples were "clean" they moved on to another section; if not, more cleaning was done until all the samples were clean. Needless to say, the chlorine content was pretty high for awhile, but at the same time it was high enough that individual homeowners did not have to do anything special to sanitize the system at their end.

Cleaning homes was a totally different story. The first step was to remove the mud and yuck that was left behind. Then the carpets had to come up and the floors thoroughly scrubbed with a disinfectant--usually chlorine bleach. In most cases, the watersoaked and crumbling wallboard had to be stripped off the walls and the insulation removed. Again, disinfectants were applied and in most cases a liberal coating of baking soda to reduce the odors which ranged from stagnate water smells to raw sewage smells.

Anything electrical had to be thoroughly cleaned--even the outlets had to be taken apart and cleaned if they had been underwater. I still don't understand how it happened, but most people reported that the outlet boxes were full of mud. I would have thought that it would have emptied out when the water receded, but apparently it didn't.

Appliances such as washing machines, dryers, freezers, furnaces, etc., simply had to be discarded. It was just too expensive to have the motors, etc. cleaned or replaced. You would think that water heaters, having no moving parts, would have been okay, but they weren't. Most of them had to be replaced also because of the mud and slime that got into the combustion chambers. Those few people who had electrical water heaters were able to clean theirs sufficiently to continue using them.

Fresh foods and in fact anything that was not canned, had to be discarded. Canned foods had to be washed and disinfected before they were useable. The trick was in knowing what was inside--most of the labels were washed off!

In the countryside, rural wells were all treated the same--they were assumed to be contaminated and were chemically sanitized. This procedure also allowed the farm water systems to be sanitized at the same time. High enough levels of sanitizers were used so that very little testing had to be done.

Septic systems did not seem to be much of a problem as long as the leeching fields were still intact. I guess the concern was that the leeching fields would become plugged, but apparently that did not happen.

In Iowa, we are not done with the clean up yet, but we have a good start on it. The weather of late has been beautiful and our spirits have been restored. There is a sense that Des Moines and Iowa will be a better place for all this adversity. It has given us the opportunity to evaluate our infrastructure and through improving it, we can improve the quality of life here.

For us, two questions remain: What will the fall rainy season be like? and When will the killing frosts arrive? And, . . . what about winter?

IAMFES

International Association of Milk, Food and Environmental Sanitarians, Inc.

MEMBERSHIP APPLICATION

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Dairy, Food and Environmental Sanitation, Vol. 13, No. 10, Pages 565-567 (October 1993)

A Guideline for Evaluating the Effectiveness of Foodservice Worker Training/Certification

Albert Metts, Dr.P.H., University of Wisconsin-Eau Claire, Eau Claire, WI 54702-4004
Vay Rodman, Dr.P.H., University of Wisconsin-Whitewater, Whitewater, WI 53190

ABSTRACT

With the current interest in developing mandatory local and state foodservice worker training/certification programs, evaluation of the programs for effectiveness is frequently desired and sometimes required by legislation. Whether required by law or not, if properly conducted, evaluations of training/certification programs can be helpful to food protection program managers when extended to include the degree to which training/certification may be a positive factor in compliance scores.

This paper suggests evaluation methods for this purpose and discusses four alternative outcome measures with their attendant advantages and disadvantages. Methods of controlling for bias and establishing baseline sanitation levels are also discussed.

Several states have enacted mandatory foodservice certification laws that require one or more manager or foodservice worker to be certified as having basic knowledge of food protection. The passing of a written examination and/or the completion of a training course usually precedes the official certification. If a state law has an evaluation requirement for determining the effect of the training and certification on regulatory compliance, it is necessary to make appropriate plans for conducting such evaluations. A recently enacted law in the state of Wisconsin has such a requirement (1). However, if a law is silent on evaluation, properly conducted evaluation studies can be very helpful to food protection program managers by providing vital information concerning the degree to which training may be a factor in accomplishing predetermined program outcome objectives.

Although no method exists that shows with absolute certainty the degree to which a program activity caused a program outcome, several methods can be used which provide program managers with information that is likely to be important in making decisions concerning program effectiveness.

Methodology

If possible, a control group should be identified and included in the study. In studies relating to training/certification and compliance levels, such a group would consist of those establishments whose managers or workers have not received the requisite training. If training has been conducted among one group of foodservice workers (experimental) but not among others (control), the differences in inspection scores can be measured before and after the training has been conducted. Pre- and post-inspection score differences observed in the experimental group can be compared to the respective inspection scores observed in the control group.

Considerable caution should be exercised, however, in the selection of those to be included in the experimental group in retrospective studies. For example, if a legal deadline for mandatory certification has been established, those from the establishments that tend to show higher inspection scores may become certified earlier than those from the lower scoring establishments. If the behavior of those from the higher scoring establishments is more likely to change as a result of the training than those from the lower scoring establishments, the differences in the pre- and post-inspection scores may very well show results that would not be representative of the entire population of foodservice personnel for any particular area. When a retrospective study is conducted, it is always important that the experimental group be truly representative of all those who will become certified and not the sub-group alone that may seek early certification. In this same regard, efforts should also be made to control for instructional differences, if any, in the experimental group. Two or more training programs within a study area that vary significantly in content and/or instructional emphasis can be a major source of error.

Problems associated with subjects' recall is another possible source of bias in retrospective studies; therefore, if a questionnaire is used to obtain the date of certification directly from the foodservice worker-manager/establishment, differences in pre- and post- inspection scores should be interpreted with caution. Data collection from a central source, such as a certifying agency, can frequently control for recall bias of this kind.

Prospective studies do not have the inherent problems of potential bias that retrospective studies have but they usually require much more time to complete and therefore may suffer from attrition (e.g. establishments that go out of business or change ownership). In places where mandatory certification laws require certification by a certain date, prospective studies that commence early enough and continue to the target certification date and well beyond would appear to hold the most promise of providing reliable study results.

It is also important to evaluate the training courses and examinations to determine the degree to which the specific elements of instruction and associated examination questions are linked to the sanitation standards on which the inspections are based. For example, if little or nothing of a practical nature is included in the training relating to warewashing, one could expect associated violations on subsequent inspections in those establishments that practice it. Also, mandatory certification laws may carry with them unwritten legal responsibilities which could, at best, lead to a lack of legislative support and, at worst, result in legal actions against certifying bodies. Such has been pointed out by the National Conference on Food Protection (2):

> *If we allow certification to take place without the demonstration of a specific level of competency, we are, in effect, misleading the public. On the other hand, if the required levels of competency are not valid and reflective of the practical technical competency required, we are also misleading the public and the certification candidates. We must take steps to insure that our certification is not a masquerade open to legal action. The certifying body can be sued.*

Certification without verification through appropriate evaluations could engender a false sense of security by the public in much the same way that "heath examinations" of foodservice workers may have done several decades ago.

Baseline Data

As with most public health programs, in order to determine the degree of achievement, baseline data must be known for both experimental and control groups. Food protection programs are usually evaluated for effectiveness by determining the sanitation compliance level, which is frequently based on numerically weighted scores of all establishments or subgroups within a jurisdiction. The establishment of baseline data is the first essential step in conducting effectiveness evaluations and the basis for it is a valid and reliable method of measuring the results of each official inspection.

If inspections performed by regulatory agencies have not been standardized among the inspecting staff (including those who conduct surveys of local agencies and act as agents for a state regulatory agency), no valid conclusions can be drawn about accomplishments, especially when one is attempting to measure the effect of only one dependent variable, such as foodservice manager training.

It is possible, however, to evaluate the effectiveness of foodservice training programs according to one or more indicators of outcome, depending on the program objectives. If program outcome objectives, such as improved inspection scores, are established rather than process objectives, more than one method is available for evaluating outcome. If the program objective is related to the reduction of foodborne diseases, it is simply a matter of counting the number of reported outbreaks and cases before and after the commencement of training and assessing the difference and any confounding and extraneous factors, such as changes in reporting and enforcement actions.

Other outcome indicators which can be used to measure degree of compliance are: (1) the number of unweighted violations, (2) the percent of compliance based on weighted violations (0-100%), and (3) the number of conditions or percent of compliance with the Hazard Analysis Critical Control Point System (HACCPS) (3).

Although each indicator has certain advantages and disadvantages, as shown in Table 1, there are compelling arguments for evaluating a program on two or more of the above. Doing so would be analogous to determining the health status of a community by evaluating such indicators

Table 1
Evaluation for Effectiveness of Foodservice Certification:
Advantages/Disadvantages of Alternative Outcome Measurements

Outcome Measurement	Advantage	Disadvantage
Change in Incidence of Foodborne Diseases	Easy to perform	Underreporting of foodborne diseases Surveillance and reporting would need improvement Not a good indicator of the overall sanitation level
Number of unweighted violations	Relatively easy to perform A measure of general sanitary conditions	All violations have equal value Large establishments may have greater probability of achieving lower scores than smaller ones Not specifically oriented toward foodborne disease prevention
Percent Compliance based on weighted violations (0-100)	Relatively easy to perform Already being done in some jurisdictions Well understood A measure of general sanitary conditions	Large establishments may have greater probability of achieving lower scores than smaller ones Not specifically oriented toward foodborne disease prevention
Hazard Analysis Critical Control Point System (HACCPS)	Oriented toward foodborne disease prevention	May be more difficult to provide effective training Application rate may be minimal in smaller establishments More time required by enforcement authorities Less emphasis on structural conditions, housekeeping, etc.

as morbidity, mortality, and indirect measures (educational attainment levels, housing conditions, income and so on). Moreover, for meaningful comparisons, establishments can be grouped for evaluation purposes according to the mean number of meals served per day, volume of food processed, types of menus, or level of hazards.

Pre- and post- examinations are frequently given in foodservice sanitation training courses but they are only indicators of cognitive learning based on written examinations and should only be considered process measures and not outcome (behavior change). The ability to pass a certification examination is not the purpose of certification programs. The main purpose is behavior modification which should lead to improved food protection.

Summary and Conclusion

In order to determine the effect, if any, that training/certification programs have on compliance with food protection regulations, baseline compliance levels based on one or more method for recording inspection results should be established. Once the baseline levels of compliance are established, evaluation of the training/certification program can be made with the selection of an experimental (those trained/certified) and control (those who have not been trained/certified) group. Pre- and post- inspection scores of the two groups can then be analyzed. It is important, however, to control for bias, the method of which may be determined on whether the study is prospective or retrospective in nature. Where more than one training course or certification examination exists within a jurisdiction, it is important that any significant differences be identified and, if possible, controlled.

Acknowledgments

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Legionella: An Environmental Concern

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THE PUZZLE

In 1976 there was a mysterious outbreak of disease in Philadelphia which infected many of those who had attended an American Legion convention from July 21-24. There were 180 of the Legionnaires who became infected with respiratory illness, 29 of whom died (9). 0f those infected, "140 were males, 63 were known to have preexisting illness, such as cardiopulmonary disease, diabetes mellitis, and malignancy. The case-fatality ratio was 29% for those with preexisting illness and 5% for those without" (8). The symptoms of the infected persons were "an acute onset of fever, chills, headache and malaise, followed by dry cough and myalgia." Some of the worst affected "developed high fever and died in shock with extensive pneumonia" (6).

INITIAL WORK

Intensive research was begun immediately to discover the bacterium responsible and the source of the outbreak. A formal survey completed by the Legionnaires proved that most of the victims of the epidemic had lived at the same hotel during the convention (7). The relationship of their rooms to one another was investigated and the cases "showed no unusual pattern of occurrence" (8). It was also found that "neither food nor drink [could] be significantly implicated as sources of the disease agent" (8).

Another hypothesis was that chemical poisons could have been a cause for the illness. Tissue samples from several cases were tested by neutron activation for "metals and organic toxicants." The resulting values were found to be at normal levels (7). The case was beginning to look dim. It seemed that no one would be able to identify what the disease was or where it had come from.

ISOLATING THE CAUSATIVE AGENT

Almost six months of extensive research and laboratory testing passed unproductively until mid January 1977. The bacterium responsible for causing Legionnaires' Disease "was first isolated from the lung tissues of 1 fatal case of Philadelphia respiratory disease and 1 fatal case of Broad Street pneumonia" (10). The bacterium, which was later named *Legionella pneumophila*, had not previously been isolated and the actual source of the bacterium remained a mystery. (The Broad Street case refers to 38 cases of the disease found in people within one block of the Legionnaire's hotel around the time of the outbreak in 1976. They had no involvement with the Legionnaire's convention nor had they entered the hotel) (10).

After the isolation of *L. pneumophila* it was possible to correctly test other cases for its presence. It was then discovered that an outbreak of Legionnaires' Disease occurred in 1966 at St. Elizabeth's Hospital in Washington, DC. Blood sera had been stored from 14 of the 94 patients (16 of whom were fatalities). From these sera samples antibodies to *L. pneumophila* were identified (10).

The Center for Disease Control had saved sera from 37 cases of an outbreak affecting 144 people in Pontiac, Michigan, 1968 (previously called Pontiac Fever). Thirty-two of these indicated that the outbreak was caused by the same antigen responsible for the Philadelphia epidemic — *L. pneumophila* (1).

THE ENVIRONMENTAL SOURCES

Finally, in August 1978, the environmental source of Legionnaires' Disease was discovered. The bacterium was isolated from a sample of water taken "from an air conditioning cooling tower" associated with an outbreak in Bloomington, Indiana (2). The tower was located on top of the "Indiana Memorial Union — a hotel student union complex in which 19 of 21 confirmed cases had stayed overnight" (2). (A creek near the IMU also tested positive for the bacterium.) How does someone become infected when the bacterium is in the air conditioner cooling system? When an air conditioning system is turned on, the bacterium is transmitted to the person via the air. Since the cooling systems are used primarily during warm months, there is a relative seasonality associated with Legionnaires' Disease. These cases proved that *L. pneumophila* had in fact been active prior to the Philadelphia incident in 1976, though it had not been isolated until 1978. The cases had been attributed to pneumonia whose symptoms are similar to those of Legionnaires' Disease.

Since the 1976 Philadelphia outbreak, much has been discovered about Legionnaires' Disease and *L. pneumophila*. *L. pneumophila* is a naturally occurring organism found in warm, stagnant water. The bacteria encourage the growth of a slime layer around itself to be used as nutrient and protectant. The disease is spread the infected water is aerosolized and breathed in. Person to person infection cannot take place. Some people may have a sensitivity to developing the disease due to "older age, immunosuppres-

sion, cancer, diabetes, alcohol abuse, cigarette smoking, renal disease or construction work as an occupation. . . . male-to-female ratio [is] approximately 25:1" (5).

Sources other than air conditioner cooling towers. These include whirlpools, showers, humidifiers, hot water heaters and respiratory therapy equipment. Produce misters have also been indicated as spreading the Legionnaires' Disease bacterium but it should be noted that "the concerns of legionellae in grocery stores should be directed toward breathing contaminated bioaerosols, not from consumption of food and water" (4).

LEGIONNAIRE'S DISEASE vs. PONTIAC FEVER

There are two distinct types of Legionnaires' Disease. These are Legionnaires' and Pontiac Fever. Both are spread through water and have relatively the same symptoms. Epidemic outbreaks of both types occur but sporadic cases occur only in the case of Legionnaires' Disease. Pontiac Fever unlike Legionnaires' Disease is self-limiting and non-fatal — the patient usually recovers within 2-5 days without any external treatment.

Not only is the duration of illness shorter but so is the incubation period — this is the time lapse between exposure to an organism and the development of the disease. After exposure to the bacteria, a person develops Pontiac Fever in 5-66 hours, more often than not in 24-48 hours, while for Legionnaires' Disease it is 2-10 days, most likely 5-6 days (3). The infection rate of the different types vary greatly, also. Of those exposed to the Legionnaires' Disease bacterium, 1% will develop the disease (10-20% fatalities) while 95% of those exposed to the Pontiac Fever bacterium will develop the disease though without fatalities (5).

COMMUNITY ACQUIRED vs. NOSCOMIAL INFECTION

There is also a distinction made between community acquired and nosocomial cases of Legionnaires' Disease. Community acquired cases occur when a person is exposed to the *L. pneumophila* somewhere in the environment of their community, Nosocomial cases occur when a person is exposed to the bacteria in a hospital while there being treated for something else. The two types differ in that nosocomial cases infect a population which is more susceptible, the cases tend to be more severe and there is a larger number of fatalities (25-55%). The greatest reason for these differences is that the patients who acquire nosocomial Legionnaires' Disease are usually immunocompromised which is often due to immunosuppression therapy used after organ transplants. Legionnaires' Disease differs from other nosocomial disease in that it is spread by the hospital environment rather than by people in the hospital (11).

CONTROL

It is difficult to eradicate *Legionella* from a water system but it can be done through the hyperchlorination and superheating of the system. Hyperclorination (2-6 ppm) is, however, "expensive and causes corrosion of the distribution system. Superheating is less expensive and easier to implement. The water temperature must be raised above 60 C to kill the organism" (11). It is beneficial to flush the system simultaneously. These treatments can prevent epidemic outbreaks from occurring, but sporadic cases cannot be controlled.

There is much controversy over whether hospitals should test their water supply for *L. pneumophila* and then implement expensive treatments. One side is that these tests are not needed unless a hospital has questionable nosocomial cases. The other side is that the water supply should be tested because Legionnaires' Disease is a very often overlooked infection. This view "is supported by a prospective study of pneumonia conducted at a hospital that until the time had not reported a single case of Legionnaires' Disease . . It was found that this organism caused 30% of nosocomial pneumonia cases on head-and-neck surgical wards" (11).

Though the chance of contracting a case of fatal Legionnaires' Disease is rather small, anyone with severe pneumonia should be tested for this disease and treated promptly.

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Dietary Risk Factors for Infant Botulism "Diet and Infant Botulism"

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ABSTRACT

Infant botulism results from the ingestion of Clostridium botulinum spores with subsequent germination and colonization of the organism in the gut and the eventual in-vivo production of toxin. The clinical effect of the toxin presents itself as hypotonia and a descending, symmetric, flaccid paralysis. Although infected infants may require supportive care (e.g. mechanical ventilation and enteral nutrition) a complete recovery is expected in the absence of complications. Factors, such as method of feeding, that contribute to the susceptibility of C. botulinum colonization of an infants gastrointestinal tract remain unclear as the majority of infants, as well as older children and adults, demonstrate no ill effects from the ingestion of C. botulinum spores. The major source of Clostridium botulinum spores is the environment as these spores are ubiquitous in soil and dust. Therefore, the potential for exposure and ingestion exists for all persons including infants. Clostridium botulinum spores have been identified in honey consumed by infants who subsequently developed infant botulism. With honey being a potential source of C. botulinum spores, as well as a documented risk factor for infant botulism, it has been recommended by public health officials to avoid feeding honey to infants less than one year of age.

INTRODUCTION

Dietary factors linked to infant botulism include method of feeding (i.e. breast milk versus formula) and exposure to honey and corn syrup. Infant botulism was first reported as a recognized illness in 1976 (1). However, an investigation conducted by Arnon et al (2) revealed a misdiagnosed case of infant botulism in the records of the California Department of Health Services of 1931. *Clostridium botulinum* although designated as a single species, is actually composed of four biologically dissimilar groups of clostridia grouped I to IV. These groups are linked by their shared ability to produce potent neurotoxins with apparently identical pharmacological modes of action and are arbitrarily classified A through G (3). Group I strains produce A, B, or F type toxins; Group II strains type B, E, or F; Group III type C and D toxins; and Group IV produces toxin type G only (4). The majority of cases involving infant botulism are associated with Group I and neurotoxins A or B (3). Cases involving types C, E, F, and G, although rare have also been reported (5,6,7,8,9,10,11,12).

Infant botulism results from the ingestion of *C. botulinum* spores with subsequent germination and multiplication in the gut and production of botulinal toxin in-vivo (13). The other two forms of botulism include: food-borne botulism which results from the intake of preformed toxin in foods and wound botulism resulting from *C. botulinum* infection and subsequent toxin production in traumatized tissue. The botulinal toxins irreversibly block the release of acetylcholine via interference with calcium-dependent exocytosis of the acetylcholine vesicles at the ganglionic synapse, post ganglionic synapse, and neuromuscular junctions (14). Return of function requires regeneration of terminal motor neurons and formation of new motor end plates (3).

The clinical symptoms of infant botulism include the development of constipation followed by progressive weakness proceeding in a symmetric descending fashion resulting in: a weak cry, diffuse hypotonia, ptosis of the eyelids, decreased gag reflex; dysphagia, weak suck, head lag, limb weakness, feeble respiratory effort and diminished or absent deep tendon reflexces (3,15,16). Although the presence of typical symptoms and electrodiagnostic abnormalities (e.g. electromyography) are used to substantiate suspicion of infant botulism, the diagnosis is confirmed by the presence of the organism in feces or toxin in blood or feces of the infant (15,17). Treatment typically consists of supportive care with particular concern for respiratory and nutritional needs. The efficacy of using antitoxin and antibiotics has not been demonstrated and generally are not used (13,15,18,19). In the absence of complications, full recovery is expected. The hospitalized case fatality rate is approximately 2%.

BREAST VERSUS FORMULA FEEDING

The method of feeding (i.e. breast milk vs. formula) has been investigated as a potential risk factor for the development of infant botulism. Attributes of breast milk believed to provide protection against *Clostridium botulinum* include: the immunologic components (e.g. secretory IgA); the influence of breast milk on gut flora and environment; and the possible presence of substances other than immunological factors that enhance development of the infant or offer protection against growth of *Clostridium botulinum* in vivo (20,21). The growth of *C. botulinum* is known to be inhibited by such factors as low pH, availability of nutrients, presence of volatile fatty acids, and presence of competitive microflora including *Bacteroides* sp., *Bifidiobacterium*, coliforms, enterococcus, and other clostridia (22,23,24,25,26). The gut

environment of breast fed infants is characterized by the presence of fermentative organisms such as *Lactobacillus* and *Bifidobacteria*, a low ph, and increased amounts of iron free lactoferrin. While that of a formula fed infant is a more neutral pH and is predominated by putrefactive flora, primarily anaerobes (e.g. coliforms and enteroccus) and *Bacteroides* sp. (21,27).

A number of studies have identified breast feeding as a risk factor for infant botulism. A two year prospective case-controlled study by Spika and colleagues (28) identified breast feeding as a risk factor for infants greater than two months of age. The dietary history of 12 cases of infant botulism in Utah between 1977 and 1979 revealed that 11 of 12 were predominately breast fed (i.e. feeds were breast milk > 90% of the time) (16). An investigation of 44 cases of infant botulism by Long et al.(29) identified breast feeding as a risk factor for infant botulism. All (100%) of the case infants, in their study, were taking breast milk as the exclusive or main source of nutrition as compared to 63% of case matched controls. However, 29 of the 44 infants had been started on formula or solid infant foods within four weeks of the onset of symptoms suggesting that the presence of transitional microflora, resulting from a change in diet, may have contributed to conditions that were favorable for the colonization of *C. botulinum* (30,31,32).

Arnon et al. (33) suggested that the ingestion of breast milk does not provide complete protection against infant botulism rather that it may attenuate the course of the illness. In their investigation 66% of the hospitalized cases of infant botulism were receiving breast milk at the onset of illness. In contrast of the 10 cases of sudden infant death (i.e. infants who were not hospitalized) attributed to infant botulism eight were exclusively breast fed iron supplemented formula while two were initially breast fed for two and three weeks respectively, but had since consumed iron supplemented formula ($\geq$10 weeks). In addition, there was a significant difference in age at the time of illness (i.e. breast fed: 13.8 weeks versus formula fed 7.6 weeks) for case infants. The significance of the additional iron in the formulas was believed to be attributed to the popularity of using this type of formula versus the potential for a growth stimulating effect of the iron as nine cases of sudden infant death not attributed to infant botulism, identified in this study, had also consumed iron supplemented formula.

HONEY AND CORN SYRUP AS A DIETARY RISK FACTOR

A study by Arnon et al. (34) involved the investigation of 41 cases of infant botulism in California between March 1976 and June 1978 and 33 hospitalized cases in 16 other states as well as one case that occurred in England between October 1977 and June 1978. It was determined that honey was significantly associated with type B infant botulism. In the California cases, 29.2% of hospitalized patients were exposed to honey prior to diagnosis with an increase to 34.7% for all cases. Further analysis of this data (35) identified five cases in which the same type organism or toxin, namely type B, was found in the sample of honey obtained from the infant's source container as well as that identified in the stool of the infected infant. All 15 corn syrup samples examined were negative for *C. botulinum* including eight samples obtained from containers fed to infants who developed infant botulism. In addition, the intake of corn syrup was epidemiologically determined to afford a protective factor against the development of infant botulism, suggesting that if corn syrup was used for a sweeter honey was not.

The investigation of 12 cases of infant botulism in Utah from March 1977 to October 1978 revealed seven had exposure to honey (16). Two of the seven honey samples contained the same type of organism which corresponded to the type of illness experienced by the respective infant. *C. botulinum* was not isolated from other commercial and home-canned foods tested which had been fed to the infants.

Spika et al. (28) reported on a two year prospective case control study of 68 laboratory confirmed cases of infant botulism from April 1985 to April 1987 reported to the CDC. Analysis for food items revealed a significant difference between groups for exposure to honey, for case infants, associated with an increased risk for case infants during the month prior to the onset of symptoms. The ingestion of corn syrup was not a risk factor for infants as a whole, however it was a risk factor for infants two months of age or older.

A case of infant botulism in Canada associated with honey intake identified type A organism in the suspected honey sample and type A toxin in the infected infants stool (36). Noda et al. (37) described a case of infant botulism in Japan linked to honey in which an approximately two month old male infant was "occasionally" fed homemade apple juice containing honey. The infant had otherwise been half breast-fed and half formula-fed since birth. Samples of the patients feces contained type A organisms and toxin. Type A spores were also found in the remnant honey consumed by the infant and on nipples from bottles. Samples of the parent's and sibling's stool; yard and potting soil; and dried milk powder consumed by the infant, were all negative for the organism.

HONEY AND CORN SYRUP AS A POTENTIAL SOURCE OF SPORES

The evaluation of 396 food items by Arnon and co-workers (34) identified *C. botulinum* in 10% of 90 honey samples. The estimated number of *C. botulinum* spores in the nine samples ranged from five to 80 per gram. No spores were identified from the other food items (e.g. breast milk, commercial and homemade canned and jared baby foods, jelly, etc.). A survey conducted by Sugiyana et al. (38) examined 55 honey samples from nine states representing 53 lots for retail sale and 186 honey collections from 154 producers. Eighteen (7.5%) of the 241 samples contained *C. botulinum* spores. The occurrence of spores in these samples ranged from two to seven per 25 grams. Huhtanen and co-workers (39) found 11 of 80 (14%) of honey samples obtained from local processors representing both foreign and domestic stock were positive for *C. botulinum*.

In Japan, the examination of 505 honey samples from 1986 to 1987 revealed *C. botulinum* spores of various types (A, C, E, and F) in 27 (5.3%) of the samples (40). Nakano

and Sakaguchi (41) reported on the presence of *Clostridium botulinum* spores in one of six samples obtained from retail shops in Hiroshima, Japan. Subsequent investigation of five additional lots (total of 17 samples) of the same brand that contained *C. botulinum* spores revealed 10 additional positive samples. The 10 samples also contained type F spores with a concentration ranging from one to 60 spores per gram. In contrast to the previously mentioned studies a survey of 122 retail honey samples, originating from 16 countries excluding the United States, on sale in the United Kingdom revealed no positive samples for *C. botulinum* spores (42). These results may explain in part the low incidence of infant botulism in the U.K.(42,43).

A survey of *C . botulinum* spores in food purchases in the Washington, D. C. Metropolitan area, by Kautter et al. (44), detected spores in two of 100 samples of honey and in eight of 40 corn syrup samples. The evaluation of 770 other food products (e.g. dry infant formula and cereal, nonfat dry milk, pasteurized whole milk, commercially canned fruit and juices, sugar, and fresh cooked carrots revealed no *C. botulinum* spores. A subsequent nationwide evaluation of light and dark corn syrup samples collected from 64 metropolitan areas representing 36 states, detected five positive samples for *C. botulinum* out of 961 total samples. However, an FDA investigation to assess the incidence of *C. botulinum* spores in corn syrup found no spores in 625 corn syrup samples (354 light and 271 dark) or in the 113 products tested that contained corn syrup (e.g. pancake, maple and table syrup) (45). In addition an investigation in Canada revealed no *C. botulinum* spores in 43 corn syrup samples (36).

ENVIRONMENTAL FACTORS

A number of studies have implicated the environment as the source of *C. botulinum* spores in cases of infant botulism. One study identified three cases of infant botulism type B associated with type B isolates obtained from soil surrounding the homes and one case of type A infant botulism found type A organism in the surrounding soil, vacuum cleaner dust, and cistern water (46). Spika et al. (28) identified living in a rural area or on a farm as a significant risk factor for infant botulism in infants less than two years old. Long and co-workers (27) investigated 44 cases of infant botulism where only six infants had prior exposure to honey. The microbial examination of samples of both honey and corn syrup consumed by six of the infants were negative for *C. botulinal* spores as were all other food samples (e.g. jelly, fruit, vegetables, and juice). However, *C. botulinum* spores were detected in more than 75 % of the infant's home environments. Arnon et al. (34) identified a case of infant botulism involving a child with type B illness who was exposed to tea containing mint leaves and type B spores were found in the soil at the base of the mint plant.

The major source of *Clostridium botulinum* spores is the environment as these spores are ubiquitous in soil and dust. Therefore, the potential for exposure and ingestion exists for all persons including infants. Factors which result in the germination of spores and subsequent toxin production in the gut of one infant but not another remain unknown, although factors such as infections dose or possible variations in virulence and toxigenicity among different strains has been suggested (47). Sakaguchi et al. (39) demonstrated a difference in the ability of particular strains of *C. botulinum* to colonize the intestinal tract of infant mice as well as produce toxins of varying antigenicity and molecular size of the toxin component.

CONCLUSION

With microbiological issues being one of the three areas of focus by federal agencies regarding food safety in the present decade (48), it would seem prudent to control exposure to potential food sources, namely honey, implicated in infant botulism. This has been the case as governmental, medical, and commercial groups have advised against the feeding of honey to infants (3, 18, 34). Present federal regulations focus on *C. botulinum* in low acid canned foods however, the presence of *C. botulinum* in other foods is not a violation. Because of this the FDA had advised against the use of honey and corn syrup for infant feedings (49). It has been recommended that warning labels on epidemiological and microbiological implicated food items may help prevent feeding of these items to infants (28). This plan would be an example of a proactive strategy with respect to the risk communication process (50) and may serve as a step in the right direction toward merging the food safety concerns of the public with that of the food regulatory agencies through improved public education.

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News

New Dictionary of Food Microbiology Now Available

Food microbiology plays an increasingly important role in food processing, quality assurance, sanitation, biotechnology, and product development, as well as public health, toxicology and nutrition.

Now, a new reference book, *Dictionary of Food Microbiology*, provides complete, expert definitions of more than 1,500 terms in the current vocabulary of food microbiology.

Written by Hanns K. Frank, Ph.D., Head, Biological Institute, German Federal Board of Nutrition, the text includes detailed discussion of the relevance and use of many of the terms defined — such as ingredients, microorganisms, foods, processes, and equipment.

Over 60 tables and figures illustrate the text, providing additional reference data and schematics of processes and line drawings of microorganisms.

The purpose of the volume is to provide a wide range of food industry personnel with a convenient, easy-to-understand reference to the materials and processes of today's food microbiology. The author is a recognized authority in the field who has held high-level positions in teaching, research, and industry.

Dictionary of Food Microbiology. Author: Hanns K. Frank, Ph.D. ISBN: 1-56676-010-0, 1993, 290 pages, 6x9, hardcover, $75.00.
Available from Technomic Publishing Company, Inc., 851 New Holland Avenue, Box 3535, Lancaster, PA 17604, U.S.A. Telephone: 717-291-5609, Fax: 717-295-4538. A detailed brochure describing this book is available from the publisher upon request.
BOOK REVIEW EDITORS: A limited number of copies are available for full-length critical reviews. For review copies, please contact Alicia Bowers, Marketing Specialist.

Bacterial Heat Resistance Unchanged in Ultrafiltered Milk

Some bacteria exhibit greater heat resistance in evaporated milk and other concentrated milk products than in unconcentrated milk. That's not true for ultrafiltered milk, research at the College of Agricultural and Life Sciences has shown. Heat treatments that work on unconcentrated milk should work just as well on ultrafiltered milk, according to E.H. Marth, emeritus professor of food microbiology at the University of Wisconsin-Madison.

Research has shown that products made by reverse osmosis or by evaporation, such as evaporated milk, may protect some bacteria against heat inactivation. Reverse osmosis and evaporation concentrate both solids and solutes. Higher solute levels, rather than higher solids content, probably increase heat resistance of bacteria in concentrated milk, Marth says.

Ultrafiltration concentrates solids, but not solutes. The process forces milk through a membrane that allows water and small molecules, such as dissolved salts, to pass, but holds back milk solids and other large molecules.

"Ultrafiltration to reasonable levels doesn't create a problem with heat destruction of microbes. Heat treatment should work as well for ultrafiltered milk as for unfiltered milk," Marth says.

Marth and J.L. Kornacki studied *Salmonella senftenberg* 775W, a heat-resistant strain of the bacterium that causes foodborne illness. A heat treatment that inactivates this strain should also kill other Salmonella strains.

The researchers also looked at two harmless but heat-resistant microbes: *Enterococcus faecium* and *Micrococcus freudenreichii*. *E. faecium* occurs normally in healthy people; it is more heat-resistant than *Streptococcus agalactiae*, a pathogen that sometimes occurs in raw milk. *Micrococcus freudenreichii* has been used in many pasteurization experiments.

They exposed the bacteria to temperatures in the heat-treatment range — from 140 F to 160 F (60 C to 68 C). (Instead of pasteurizing, cheesemakers may heat-treat milk at 150 F for 15 seconds, for example.) Overall, the bacteria they tested showed no significant differences in heat resistance in skim milk, whole milk and ultrafiltered 4X retentates from skim milk and whole milk.

Results were similar in diafiltered milk, in which water was added to the retentate, and the retentate ultrafiltered again.

The dairy industry uses ultrafiltration to concentrate and fractionate whey, to preconcentrate skim milk for drying, and to concentrate milk for cheesemaking. For cheesemakers, ultrafiltered milk gives higher yields and more uniform cheese, requires less rennet, produces less acid whey, and reduces costs for refrigeration, pasteurization and heating.

J.L. Kornacki is now director of Silliker Laboratories of Wisconsin in Madison.

Manufacturer of the Year

At the annual FISA (Food Industry Suppliers Association) Conference, Fristam Pumps was named Manufacturer of the Year. This award is presented when the membership feels that a manufacturing supplier's contribution in the area of distribution support, quality of products, services, and promotional activities is worthy of recognition.

Fristam Pumps would like to thank the members of FISA for this great honor. We pledge to continue providing our distributor partners and customers with award winning quality products and service.

Fristam Pumps, Middleton, WI, manufacturers sanitary centrifugal and positive displacement pumps for the food, dairy, beverage, pharmaceutical, and biotechnology industries.

For more information contact Connie Frick, Marketing Coordinator at (608)831-5001.

Allegheny County, PA Food Program Wins National Recognition

A national panel of experts in food safety recently selected the Allegheny County, Pennsylvania, Health Department's food protection program as the best in the nation and winner of the Samuel J. Crumbine Consumer Protection Award for 1993. The Award was presented at the annual educational conference of the National Environmental Health Association in Orlando, Florida, on June 30.

The Allegheny County unit was also honored in presentations at the International Association of Milk, Food and Environmental Sanitarians in Atlanta, Georgia, on August 4 and at the American Public Health Association annual meeting in San Francisco, California, on November 26.

Officers of these three public health societies were among the seven jurors who reviewed entries for the Crumbine Award, given annually to a local health department that has "demonstrated unsurpassed achievement in providing outstanding food protection services to its community."

The Allegheny County unit was cited for being highly creative and very responsive to community needs, especially in its risk based evaluation of chain restaurants and ethnic food establishments. The jury also remarked on the sophistication of the county's training programs, both for the inspectional staff and for foodservice managers, "and the whole gamut of imaginative and innovative ways you sought to realize the objectives you had set to improve food safety for Allegheny County."

The Crumbine Award, which casts a spotlight of recognition on the importance of food protection at the local level, is held in high esteem by the environmental health community. Nowhere else is the unsung work of restaurant and food store inspection recognized for the contribution it makes to the public's health and well-being.

The award is named after Dr. Samuel J. Crumbine, a pioneer in disease prevention techniques in public health. As the health officer of the State of Kansas at the turn of the century, Dr. Crumbine instituted many civic campaigns to improve sanitation in food and beverage service. He coined the slogan "Swat the fly!" which led to the invention of the fly-swatter; he also banned the common drinking cup, which inspired the development of the disposable cup industry.

In recognition of Dr. Crumbine's many contributions to public health in foodservice sanitation, the Foodservice & Packaging Institute, the trade association of manufacturers of disposables for foodservice and packaging, sponsors the Award in his honor. The Award is managed by an independent jury made up of health professionals who are recognized experts in the field of food safety.

For more information contact Charlie Felix at (703)777-7448.

Introducing the New "10 Points to Dairy Quality" Videos

The Milk & Dairy Beef Quality Assurance Program is now being enhanced by a new educational tool.

A set of videos in English and Spanish entitled "10 Points to Dairy Quality," makes it easy for herd owners and managers to convey the key drug management principles in the program to employees. Veterinarians and cooperative and proprietary dairy field representatives also can use the videos to illustrate drug management practices for clients and patrons.

Each video in the set of 10 provides an in-depth explanation of a critical control point in the Residue Prevention Protocol.

All of the videos are illustrated with on-farm, packing plant, and milk-receiving-plant scenes as well as with interviews of producers, practicing veterinarians, regulatory officials and others.

The videos, which range in length from seven to 17 minutes, are designed to be viewed by employees during breaks, or some other convenient time. They will assist owners of large herds, veterinarians, or cooperative/proprietary dairies in training milkers and producers to continue the quest for quality dairy products.

U.C. Davis, in cooperation with Agri-Education, Inc., produced the videos. The complete set sells for $100. Individual videos focusing on a specific critical control point are available for $17.50 each.

More information about the videos may be obtained from Sandra Greufe, Executive Secretary, Dairy Quality Assurance Center, Box 497, Stratford, Iowa 50249 or by calling (515)838-2793 or FAX (515)838-2788.

Federal Register

Margarine; Standard of Identity to Permit Use of Any Form of Oil of Marine Species Affirmed as GRAS or Approved as a Food Additive for This Use

Agency: Food and Drug Administration, HHS.

Action: Tentative final rule.

Summary: The Food and Drug Administration (FDA) is issuing a tentative final rule that sets forth an amendment to the U.S. standard of identity for margarine to permit the use of any form of oil from a marine species that has been affirmed as generally recognized as safe (GRAS) or approved as a food additive for this use. This action is in response to a petition submitted by the National Fish Meal and Oil Association (NFMOA). FDA believes this action will provide increased flexibility to manufacturers in the selection of lipid ingredients in margarine and will promote honesty and fair dealing in the interest of consumers.

Dates: Written comments by September 16, 1993. The agency proposes that any final rule that may be issued based upon this tentative final rule shall become effective October 18, 1993.

Addresses: Submit written comments to the Dockets Management Branch (HFA-305), Food and Drug Administration, rm. 1—23, 12420 Parklawn Dr., Rockville, MD 20857.

For Further Information Contact: Shellee A. Davis, Center for Food Safety and Applied Nutrition (HFS-306), Food and Drug Administration, 200 C St. SW., Washington, DC 20204, 202-205-5112.

Supplementary Information:

I. The Proposal

In the Federal Register of August 30, 1990 (55 FR 35439), FDA published a proposal based on a petition from NFMOA, 1525 Wilson Blvd., Suite 500, Arlington, VA 22209, to amend the U.S. standard of identity for margarine (§ 166.110 (21 CFR 166.110)) to permit the use of any form of margarine oil that has been affirmed as GRAS or approved as a food additive for this use as an additional optional edible fat or oil ingredient in margarine. Interested persons were given until October 29, 1990, to submit comments.

The NFMOA petition was filed under section 701(e) of the Federal Food, Drug, and Cosmetic Act (the act) (21 U.S.C. 371(e)), which requires formal rulemaking in any action for the amendment of a food standard. However, in November, 1990, the Nutrition Labeling and Education Act of 1990 was signed into law, and it removed food standard rulemaking proceedings, except for action for the amendment or repeal of food standards of identity for dairy products or maple syrup, from the formal rulemaking proceedings of section 701(e) of the act. Therefore, further action on the NFMOA petition is subject to the informal notice and comment rulemaking proceedings of Section 701(a) of the act. This tentative final rule is being published under those procedures. Because there was an opportunity to comment on the proposal when it was published in 1990, the comment period on this tentative final rule will be 30 days.

II. Comments to Proposal

FDA received six letters, each containing one or more comments, from trade associations, industry, consumers, and the U.S. Congress in response to the proposal. Five of the letters supported, and one letter opposed, the proposal. One of the supporting letters requested clear labeling requirements. A summary of the suggested modification and of the opposing letter and the agency's responses follows:

1. One comment expressed concern about possible allergic reactions occurring in persons sensitive to fish and fish products and requested that FDA require that any margarine made with marine oil to be clearly labeled as such.

The agency finds the concern expressed by the comment to be unfounded. First, source information is part of the ingredient's common or usual name that is required under provisions of section 403(i) of the act (21 U.S.C. 343(i)). The agency requires that such names accurately identify or describe, in as simple and direct terms as possible, the basic nature of the ingredient or its characterizing properties. This requirement is set forth in 21 CFR 102.5(a), which establishes principles for devising common or usual names for foods.

In addition, the standard of identity for margarine in § 166.110 (d) requires that each of the edible fats or oils used in the food be declared on the label in accordance with the applicable sections of 21 CFR parts 101 and 130. As provided in 21 CFR 101.4(b)(14), each individual fat or oil ingredient of a food must be declared by its specific common or usual name (e.g., "beef fat," "cottonseed oil").

Finally, "hydrogenated menhaden oil" or "partially hydrogenated menhaden oil" is the name of the ingredient as defined in 21 CFR 184.1472 and must be identified as such in the ingredient statement. If FDA lists other oils of marine species as food additives for use in margarine or affirms such oils as GRAS for such use, the name of the oil will reflect its source.

For these reasons, the agency concludes that persons with allergies to fish and fish products will be adequately informed of the presence of such oils in margarine.

2. One comment raised the issue of adverse health effects from the increased use of oil derived from menhaden, whose schooling and feeding habits locate them, during growth and maturation, in areas contaminated by substances classified as toxic or hazardous.

FDA considered the safety of partially hydrogenated and hydrogenated menhaden oils during the GRAS review process (GRASP 6G0316) (54 FR 38219, September 15, 1989). After evaluation of all available information, the agency concluded that the use of partially hydrogenated and hydrogenated menhaden oil as an edible fat or oil should be affirmed as GRAS. Information concerning this review is on display at the Dockets Management Branch (address above) under Docket No. 86G-0289 and may be seen by interested persons between 9 a.m. and 4 p. m., Monday through Friday. No evidence of contamination of these oils was presented during that review. Therefore, FDA is not aware of any basis for the concern expressed by the comment. If FDA receives evidence that listed oils contain deleterious substances and are a health hazard, the agency will take appropriate action.

FDA will evaluate carefully, as part of its review of any future petitions for the listing of other oils of marine species

for use in margarine, the possibility that such oils could contain deleterious substances. The agency notes that margarine is adulterated under section 402(e) of the act (21 U.S.C. 342(e)) if any of the raw material used therein consists in whole or in part of any filthy, putrid, or decomposed substance, or is otherwise unfit for food.

III. Conclusion

The agency has tentatively concluded that it is reasonable to provide for the optional use of any form of oil or marine species that has been affirmed as GRAS or listed as a food additive for this use, and that doing so will promote honesty and fair dealing in the interest of consumers.

Accordingly, after consideration of all comments, the agency is issuing a tentative final rule to amend the standard of identity for margarine as set forth below. In addition to the proposed changes, FDA is making some minor editorial changes for clarity and is revising the format of the section.

IV. Economic Impact

FDA has examined the economic implications of this tentative final rule to amend 21 CFR part 166 as required by Executive Order 12291 and the Regulatory Flexibility Act (Pub. L. 96-354). Executive Order 12291 compels agencies to use cost-benefit analysis as a component of decisionmaking. The Regulatory Flexibility Act requires regulatory relief for small businesses where feasible. The agency tentatively concluded in the August 30, 1990, proposal, that this action would not result in a significant economic impact on a substantial number of small entities. Manufacturers may continue to produce margarine using current formulations. Thus, no changes are required in formulations unless manufacturers wish to reformulate the products. FDA has received no new information or comments that would alter the tentative finding that it set out in the proposed rule, that there is no substantive economic issue in this rulemaking, and that this is not a major rule as defined by either Executive Order 12291 or the Regulatory Flexibility Act.

V. Environmental Impact

In the preamble to the proposed rule FDA reported its finding that this action qualified for categorical exclusion under 21 CFR 25.24(b)(1), and that neither an environmental assessment nor an environmental impact statement was required. FDA received one comment on the proposed rule that claimed that this action would result in increased fishing pressure that could adversely affect the menhaden fishery, and that the agency had not considered this potential impact prior to issuing the proposed rule. Although the comment did not provide evidence to support the contention that the menhaden fishery would be adversely affected, FDA requested an environmental assessment (EA) from the petitioner. The decision to ask for an EA takes into consideration the agency's assessment of this issue as part of its previous action to affirm partially hydrogenated menhaden oil and hydrogenated menhaden oil as GRAS (54 FR 38219). The agency also has information indicating that the market for menhaden oil as a component of margarine is large, approximately 40 million pounds per year.

Based on information in the petitioner's EA submitted for the current action and on the agency's analysis of the reports on the menhaden fisheries issued subsequent to FDA approval of GRASP 6G0316 (54 FR 38219), FDA has concluded that the proposed action will not have a significant impact on the menhaden fishery. Amending the margarine standard of identity will not increase the volume of crude menhaden oil that is produced. The only change will be that crude menhaden oil will more often be retained in the United States for further processing into food grade products, instead of being shipped to Europe and elsewhere for this same use.

FDA has carefully considered the potential environmental effects of this action, including the effects on the menhaden fishery, and has concluded that the action will not have a significant impact on the human environment, and that an environmental impact statement is not required. The agency's finding of no significant impact, and the evidence supporting that finding, which includes the petitioner's environmental assessment, the finding of no significant impact for FDA's approval of GRASP 6G0316 (54 FR 38219), and a copy of "National Marine Fisheries Service Final Purseseine Landings of Gulf and Atlantic Menhaden in the 1990 and 1991 Fishing Seasons" may be seen at the Dockets Management Branch (address above).

List of Subjects in 21 CFR Part 166

Food grades and standards, Food labeling, Margarine.

Therefore, under the Federal Food, Drug, and Cosmetic Act and under authority delegated to the Commissioner of Food and Drugs and redelegated to the Director, Center for Food Safety and Applied Nutrition, it is proposed that 21 CFR part 166 be amended as follows:

PART 166 - MARGARINE

1. The authority citation for 21 CFR part 166 is revised to read as follows:

Authority: Secs. 201, 401, 403, 407, 409, 701, 721 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 321, 341, 343, 347, 348, 371, 379e).

2. Section 166.110 is amended by adding new paragraph headings for paragraphs (a) and (c), by revising paragraph (a)(1), and in paragraphs (b) and (d) the paragraph headings "Optional ingredients" and "Label declaration," respectively, are italicized to read as follows:

§166.110 Margarine.

(a) *Description.* * * *

(1) Edible fats and/or oils, or mixtures of these, whose origin is vegetable or rendered animal carcass fats, or any form of oil from a marine species that has been affirmed as GRAS or listed as a food additive for this use, any or all of which may have been subjected to an accepted process of physico-chemical modification. They may contain small amounts of other lipids, such as phosphatides or unsaponifiable constituents, and of free fatty acids naturally present in the fat or oil.

(b) *Optional ingredients.*

(c) *Nomenclature.*

(d) *Label declaration.*

Dated: July 22, 1993

Fred R. Shank,

Director, Center for Food Safety and Applied Nutrition

(FR Doc. 93-19735 Filed 8-16-93; 8:45 am)

Food and Environmental Hazards to Health

Lead Poisoning in Bridge Demolition Workers — Georgia, 1992

Bridge demolition and maintenance are leading causes of lead poisoning among workers in the United States. In June 1992, a local health department in Georgia detected elevated blood lead levels (BLLs) in four demolition workers. This report summarizes the investigation of these cases.

In February 1992, a temporary-service company was subcontracted by a steel corporation to cut apart steel beams that had been removed from a local bridge. Four men were hired; one worker, aged 54 years, began work in late February; two, aged 36 and 28 years, in March; and one, aged 24 years, in early April. All four were immigrants from Mexico; only two spoke English. The work was performed outdoors, without protective equipment or training, using oxy-acetylene flame-cutting torches.

In April, all four workers reported light-headedness and shortness of breath from the metal fumes, requiring frequent fresh-air breaks during the day. In early May, all four workers developed a variety of symptoms including headache, dizziness, fatigue, sleep disturbance, confusion, forgetfulness, arthralgia, and abdominal pain. Paper masks were provided to the workers in late May by the steel company; however, because these became blocked within hours by the accumulation of dust, the workers discarded them. The severity of symptoms intensified through June, with nausea, vomiting, constipation, weakness, shortness of breath, loss of balance, and nervousness. The 36-year-old worker left employment for 3 weeks (from mid-June through early July) because of his symptoms.

As part of an annual risk-management assessment by the steel company's insurance carrier, personal air sampling was conducted April 30 for one of the four workers; this specimen measured an airborne lead concentration of 525 $\mu g/m^3$, more than 10 times the Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) of 50 $\mu g/m^3$ for general industry. In early June, the steel company suggested BLL examinations of the workers; their BLLs, measured at the local health department, were 93, 90, 59, and 66 µg/dL for the 54-, 28-, 24-, and 36-year-old men, respectively. The workers' employment was terminated in late June on receipt of the test results by the company.

In follow-up to the BLL results, in mid-June the health department investigated each worker's household, using a standard protocol of visual inspection and portable radiographic fluorescence readings of window sills, walls, and trim; no environmental sources of lead exposure were identified. BLLs were obtained from three children who resided in the homes; all had levels <10µg/dL, which is below the CDC BLL of concern for children.

The health department recommended that the workers promptly seek medical evaluation and care; however, because they had no medical insurance and both the subcontractor and the steel company declined to assume the costs of treatment, the workers initially delayed seeking medical treatment. They subsequently contacted an attorney, who initiated worker's compensation proceedings and arranged for a local hospital to admit them for treatment. Each worker received three 5-day chelation treatments with intravenous calcium disodium ethylenediamine tetraacetic acid approximately 15 days apart. All four reported improvement but continued to experience memory deficits, arthralgias, headaches, dizziness, and/or sleep disturbances.

The health department recommended that the workers request an OSHA inspection of the worksite. Findings from the inspection of the steel company on July 15 resulted in citations for violations of the medical removal protection and worker training provisions of OSHA's lead standard. OSHA inspectors also investigated work conditions at the bridge from which the beams were removed; the demolition company was cited for excessive lead exposures (based on the construction industry PEL of 200 $\mu g/m^{3+}$), failure to provide personal protective equipment, and failure to monitor workplace conditions.

On December 14, 1992, the workers were evaluated at a university-based occupational medicine clinic. Physical examinations of three workers were normal; the 54-year-old worker was markedly depressed with evidence of neurologic abnormalities, including a strongly positive Romberg test and marked dysnomia. BLL measurements were 27, 25, 13, and 16 µg/dL for the 54-, 28- ,24-, and 36-year-old work-ers, respectively. No further treatment was recommended, but follow-up BLL monitoring was planned.

Editorial Note: An estimated 90,000 bridges in the United States are coated with lead-containing paints. Because of maintenance and reconstruction requirements, lead exposure is a continuing occupational health hazard for construction and demolition workers. Previous cases of lead poisoning associated with similar work have been characterized by extremely high BLLs in affected workers, which developed after brief exposures and, in some instances, were unresponsive to chelation therapy.

The findings in this report are consistent with other studies that indicate that minority groups are disproportionately exposed to lead and other occupational hazards. In addition, the hazardous process described in this report (flame-cutting or burning of paint-coated steel beams) had been subcontracted to a smaller company by a larger, well-established firm. Such subcontracting is common in the construction industry but often concentrates hazards among workers with limited access to appropriate training, personal protective equipment, and other safety and health measures.

Construction workers are subject to highly variable exposures, and high worker-turnover rates in the construction workforce may pose special hazards for construction workers. Effective June 3, 1993, a new interim final OSHA standard on "Lead Exposure in Construction" extends to workers in the construction trades the basic health and safety provisions of the OSHA lead standard for general industry, such as requirements for medical monitoring and medical removal protection.

The response of the health department to the lead exposure in these workers was prompt and effective. However, the limitations of the interventions available and the persistence of the workers' symptoms underscore the need for primary prevention — including portable local ventilation, personal protective equipment, personal hygiene measures, and worker training — during bridge renovation and related demolition work.

Morbidity and Mortality Weekly Report 5/28/93

Lyme Disease—United States, 1991-1992

Surveillance for Lyme disease (LD) was initiated by CDC in 1982, and in 1990, the Council of State and Territorial Epidemiologists (CSTE) approved a resolution making LD nationally reportable. During 1982-1991, states reported 40,195 cases of LD. In 1992, LD accounted for more than 90% of all reported vectorborne illnesses in the United States (CDC, unpublished, 1993). This report summarizes surveillance for LD in the United States during 1991-1992.

Forty-nine states and the District of Columbia require reporting of LD. The CSTE/CDC surveillance case definition requires the presence of an erythema migrans rash or at least one objective sign of musculoskeletal, neurologic, or cardiovascular disease and laboratory confirmation of infection.

During 1991, 47 states reported 9465 cases of LD to CDC; during 1992, 45 states reported a provisional total of 9677 cases, representing a 19-fold increase over the 497 cases reported by 11 states in 1982. Most cases were reported from the northeastern, mid-Atlantic, north central, and Pacific coastal regions. Established enzootic cycles of *Borrelia burgdorferi*, the causative agent of LD, have been identified in 19 states; these states accounted for 94% of cases reported during 1991-1992.

The overall incidence rate of reported LD during 1992 was 3.9 per 100,000 population. During 1992, Connecticut (53.6 cases per 100,000), Wisconsin (10.7), and California (0.8) reported the highest rates in the northeast, north central, and Pacific coastal regions, respectively. Rates in some counties in California, Connecticut, Massachusetts, New York, and Wisconsin exceeded 200 cases per 100,000; the incidence was highest in Nantucket County, Massachusetts (449.1). The number of reported cases in Connecticut and Rhode Island increased 48% and 93%, respectively, over 1991. New York reported a provisional total of 3370 confirmed cases during 1992, a decrease of 574 cases from 1991. From 1991 through 1992, decreases were greatest in Westchester (1762, compared with 1154) and Suffolk (860, compared with 654) counties. In 1992, these two counties accounted for 19% of the national total, compared with 28% in 1991.

Among 7507 cases analyzed for which patient age was given, the largest numbers were reported for persons aged 0-9 years (1087[14.5%]), 30-39 years (1272 [16.9%]), and 40-49 years (1271 [16.9%]). Of 7642 cases, 3770 (49.3%) occurred among males.

Editorial Note: The distribution of LD in the United States is highly correlated with the distribution of the principal tick vectors *Ixodes dammini* (reported to be the same species as *I. scapularis*, the black-legged tick) in the northeastern and north central regions and *I. pacificus* (i.e., the western black-legged tick) in the Pacific coastal states. The occurrence of sporadic cases in states without established enzootic transmis-sion of *B. burgdorferi* may be due to infectious exposures in limited, unrecognized foci, exposures during visits to areas with endemic LD outside the state of residence, misclassification, or misdiagnosis. Enzootic foci are highly localized and are dependent on environmental factors favorable to vector ticks and their maintenance hosts (especially deer) and to rodent reservoirs of *B. burgdorferi*. Therefore, subtle ecologic differences may account for substantial differences in incidence between states, counties within states, and adjacent townships.

The 19-fold increase in reported LD cases since 1982 may reflect a combination of at least four factors: heightened awareness of LD by patients and physicians; increased use of laboratory testing in LD diagnosis; increased surveillance and health department requirements for reporting; and a true increase in the number of cases. Surveillance practices in particular have had an important impact on the reported occurrence of LD. For example, active physician-based surveillance conducted in 1992 by state health departments in collaboration with CDC in Connecticut and Rhode Island resulted in substantial increases in reported cases over 1991. By contrast, the decrease in reported cases in Suffolk and Westchester counties, New York, probably reflects reductions in state and county surveillance personnel necessary to maintain previous levels of case detection and validation.

LD is considered an emerging infectious disease because of the impact of changing environmental and socioeconomic factors, such as the transformation of farmland into suburban woodlots that are favorable for deer and deer ticks. Demographic profiles of persons with LD reflect mostly suburban and rural risk. Evidence suggests both continuing geographic spread and increasing incidence over time in established endemic foci.

The diagnosis of LD is based principally on clinical findings, and results of serologic testing are supportive. Serologic tests for LD are not standardized, and problems in the reliability and accuracy of serologic test results have limited their usefulness for surveillance purposes. CDC, in collaboration with the Association of State and Territorial Public Health Laboratory Directors, held a workshop on standardized serologic testing for LD in March 1993, and an evaluation of a standardized testing protocol by selected public health laboratories will be conducted during May-August 1993.

Although the numbers of LD cases reported by some states have fluctuated by year, the annual number of reported cases in the United States has remained relatively constant during 1989-1992, possibly reflecting the implementation of the uniform case definition and standardized reporting. However, the true incidence of LD in the United States is unknown, and estimates are subject to the influences of underreporting, misclassification, and overdiagnosis. The development of standardized, sensitive and specific serologic tests and better surveillance should result in improved estimates of LD.

MMWR 5/14/93

HAZCON-Based Total Quality Management

Establishing a Hazard Control-Based TQM and Program Assessment of an Operation (Part XVI) cont.

O. Peter Snyder, Jr., Ph.D.,
Hospitality Institute of Technology and Management,
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St. Paul, MN 55114

Foodservice HAZCON-Based QA Manual

This list shows the Table of Contents of the written documentation entitled, **Foodservice HAZCON-Based QA Manual**, which is used by organizations for the TQM process. Note that the manual is laid out by organization. First, there are general procedures that all employees must follow. Then, there is food preparation, which is the foodservice group; sanitation and maintenance group; food receiving group; finally, the operator/manager. Quality-assured recipe procedures are incorporated to complete the manual.

FOODSERVICE HAZCON-BASED QA MANUAL

FOOD SAFETY QUALITY ASSURANCE POLICY

ORGANIZATION CHART OF ASSURANCE FOOD SAFETY

GENERAL FOOD SAFETY PROCEDURES AND STANDARDS FOR ALL PERSONNEL
- Personal Hygiene
  - Individual illness
  - Cuts and abrasions
  - Personal cleanliness
  - Fingernails
  - Hair restraint
  - Jewelry and hard objects in pockets
  - Handkerchiefs and facial tissues
  - Chewing gum, smoking, eating
  - Handling food in front of the customer
  - Double hand washing
  - Single hand washing
  - Food sinks vs. hand and utility sinks
- Sanitizing surfaces
  - Using sanitized equipment
  - Frequency of sanitizing food contact surfaces
  - Food contact surface wiping cloths
  - Separate cleaning and sanitizing solutions

FOOD PREPARATION PERSONS FOOD SAFETY PROCEDURES AND STANDARDS
- Cooks, food supervisors, salad persons, bakers
- Food product thermometers
- Food thawing
- Pre-preparation
  - Food washing
  - Raw food handling
  - Ingredient control
  - Separate raw and cooked food preparation equipment
  - Labeling
- Preparation
  - Food protection
  - Hard foreign objects
  - Safe preparation of multi-portion, thick, > 2-inch items
  - Safe preparation of single portion, thin, < 2-inch items
  - Sauces, soups, beverages
  - Fruits, vegetables, legumes, cereals
  - Milk
  - Bread, pastry
  - Hot combination dishes
  - Cold combination dishes
  - Food holding temperatures
  - Serving, packaging, transporting
  - Handling food, money, dirty tableware
  - Food tasting
  - Carry-out and banquet food
  - Returned food
  - Leftovers
- Storing prepared food
  - Food cooling time
  - Cross-contamination drip
  - Storage time
  - Salad bar
  - Storage containers

SERVICE PERSONS FOOD SAFETY PROCEDURES AND STANDARDS
- Dishware
- Work station cleanliness
- Separate raw and cooked food preparation equipment
- Frequency of surface sanitizing
- Beverage dispensing equipment
- Milk product dispensers
- Self-service food, dishes, utensils
- Table condiments
- Ice scoops
- Food serving temperature

SANITATION AND MAINTENANCE PERSONS FOOD SAFETY PROCEDURES AND STANDARDS
- Leftover disposal
- Detergents
- Laundry facilities

Cleaning cloths
Changing sanitizing solutions
Equipment cleaning
Food contact surface equipment
Non-food contact surface equipment
Storage of dishware
Cleaning equipment storage
Ware washing procedures
Ware washing equipment
Sink washing procedures
QC inspections
Garbage
Rest rooms
Floors, walls, and ceiling
Ventilation system

FOOD RECEIVING AND STORING PERSON FOOD SAFETY PROCEDURES AND STANDARDS
Inspection of incoming products
Substandard products
Chemicals separation
Container disposal
Stock rotation
Proper storage procedures
Pest control and materials used
First aid material

OPERATOR OR MANAGER FOOD SAFETY PROCEDURES AND STANDARDS
Evaluation of unit performance
Commitment to zero defects and allocation of resources
Food safety improvement program
Allocation of resources
Holding subordinates responsible for safety results
Quality Management Team
Coaching and skill development
Setting the example
Maintaining equipment
Reporting illness
Customer comment cards
Smoking areas
License
Ethical truth in menu practices
Design of the facility
Water

QUALITY-ASSURED RECIPE PROCEDURES (QARP)
Cooked and served items
Cooked, packaged, and stored items
Packaged, cooked, and stored items
Training
Initial training for all new employees and management
Continuing education and correct procedure reinforcement
New employee training record
Responsibilities taught
Current employee performance improvement training record
Lesson outline (for current employees)

CLEANING AND SANITIZING SCHEDULE AND INSTRUCTIONS

PEST CONTROL SCHEDULE AND INSTRUCTIONS

MAINTENANCE SCHEDULE

FOODBORNE ILLNESS INFORMATION FORM

RETAIL FOOD OPERATION FOOD HAZARD CONTROL CHECKLIST

Part of the documentation of an effective safety-assured program is employee training. Hence, the manual includes a requirement for employee training documentation.

Cleaning and sanitizing, pest control, and maintenance schedules are necessary in order to keep the facility in good order and condition.

There is a foodborne illness information sheet, which is provided so that anyone on duty who is told of an alleged illness by a customer can record data for follow-up.

Checklist

Finally, there is the master checklist used for self-control by the organization. The **Retail Food Operation Food Hazard Control Checklist** is the key to government approval of a self-control program. This checklist needs to include all procedures that the retail food operation intends to follow to ensure safety. Checklists are retained by the operation only until the operation is able to take action on any deficiency it finds. The objective is that there is no deficiency in the organization that has persisted for more than one week, provided there is a self-inspection once a week. Once all deficiencies are corrected, then the checklist can be discarded.

Government Inspection

When an organization is to be rated by a government agency, it should start with an opening conference, at which time the government agent/inspector should ask to see the employer's written food safety program. If the program is not in writing, then it should be clearly described by the owner. Then, the inspector should have a "walk-around", at which time the inspector will note whether the organization is aware of its deficiencies and has corrective action pending, or whether the organization is not in control. The inspector would then look for the following:

1. Employee job instructions prior to work assignment
2. Existence and enforcement of food safety rules
3. Training in food hazard controls and other required training
4. Use and care of food processing equipment and the facilities
5. Handling of incidents and follow-up action
6. Existence of food safety committees and meetings, and the degree to which top management enforces safety
7. Action program to improve the food safety assurance program.

The overall evaluation will be in terms of the following six items:

1. Management responsibility and commitment
2. Written program
3. Hazard analysis and control
4. Communications and training of employees
5. Problem investigation and corrective action
6. Program enforcement.

Industry Products

Difco Introduces Prepared Quality-Control Bacto® Sterility Test Bottles with Screw Cap or Septum

Two media required for quality control testing to determine sterility are now available in prepared quality control sterility test bottles from Difco Laboratories.

In addition to consistent quality and performance, Difco's Tryptic Soy Broth and Fluid Thioglycollate Medium in prepared Bacto Sterility Bottles offer laboratories a convenient, reliable and economical alternative to dehydrated media.

Difco's microbiology expertise assures confidence in test results. The media are engineered to meet strict assay standards, and conform with United States Pharmacopeia requirements for sterility test procedures (Section 71 USP XXII). The USP requires Soybean Casein Digest Medium (Tryptic Soy Broth) for aerobic testing and Fluid Thioglycollate Medium for anaerobic testing.

Difco has incorporated a number of innovative features into Bacto Sterility Bottles. They have laminated acetate labels that resist alcohol or chlorine treatment in preparation for clean-room use. The clear labels enhance visual examination of the bottles. Color-coded caps and markings on each label simplify differentiation of the media. The septum cap unscrews for access to the media and culture. Wide-mouth screw cap bottles for both media also are available.

The sterility bottles are available in shrink-wrapped tray packages of 10, which provide tamper evidence and protect the bottles from dirt and moisture.

Each package includes a Certificate of Analysis to simplify documentation. Bacto Sterility Bottles are available from a national network of distributors who sell the full line of quality Difco products.

Difco Laboratories has been a technological leader in the development and manufacture of microbiology-related products for nearly 100 years.

Difco Laboratories - Detroit, MI

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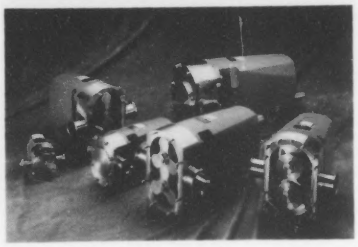

Tri-Clover T-Series Pumps Meet USDA Approval

Tri-Clover's T-Series of rotary lobe pumps, designed for a broad range of applications in the dairy, food, and pharmaceutical industries have been approved by the USDA. TSK and TSR models are approved for use in meat and poultry processing plants. In addition, the TSK Ultra-Clean Series has recently been approved by the USDA Egg Division for use on CIP'able process lines in egg processing plants.

T-Series pumps are designed to meet 3A standards for sanitary design, and are CIP compatible. All wetted parts are constructed of corrosion resistant type 316 stainless steel.

Among the applications for the pumps are the processing of suspended solids, abrasive slurries and liquid gas mixtures and the handling of high, medium and low viscosity materials.

Popular models in the T-Series include the TSR pump, available in 12 sizes for applications up to 700 GPM and viscosities up to 1,000,000 Cps, and the TSK "ultra-clean" pump for capacities of 330 GPM and pressure ranges to 220 psi.

The TSR and TSK models offer a variety of seal types and seal face materials. The modular casing design of TSR models affords a wider range of choices in pump selection, by allowing processors to vary rotor choice and casing length.

All pumps in the line are designed for operating efficiency and easy maintenance. In addition, the lack of rotor-to-rotor contact in the casing provides for durable, low-wearing performance. Servicing features include easy casing access and interchangeable rotors, seals, bearings and gears for fast repair and minimal parts inventories.

Headquartered in Kenosha, Wis., Tri-Clover Inc. is a leading manufacturer of sanitary stainless steel valves, pumps and fittings, as well as flow control, batch/weigh and Clean-In-Place (CIP) systems. Founded in 1919, Tri-Clover Inc. is now an Alfa Laval Flow Company.

Tri-Clover, Inc. - Kenosha, WI

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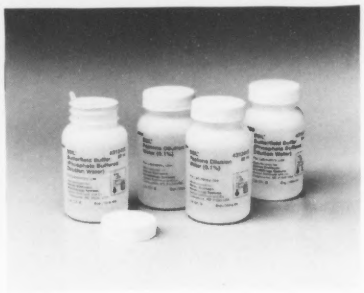

New Disposable Dilution Bottles Deliver Enhanced Laboratory Safety and Efficiency

Becton Dickinson Microbiology Systems, Cockeysville, MD, announces the immediate availability of a complete line of BBL® Disposable Dilution Bottles. The recyclable bottles include Peptone Dilution Water (0.1%) with a pH of 7.1± and Butterfield Buffer (Phosphate Buffered Dilution Water) with a pH of 7.2±, in both 90 and 99 ml fill volumes.

All BBL® dilution bottles are gamma-irradiated. Each bottle features a screw-cap lid that is easily removed and replaced, with an easy-peel induction seal that keeps the dilution water stable throughout the product's long-term room temperature storage. The extra-wide throat allows easy specimen insertion, and the unbreakable polycarbonate bottle is recyclable.

All BBL Dilution Bottles meet or exceed APHA or USP guidelines as expressed in the APHA *Compendium of Methods for the Microbiological Examination of Foods* or the USP standards for the cosmetics and pharmaceutical industry. Lot-specific certificates of analysis and gamma-irradiation are available upon request.

Unlike the lengthy and cumbersome procedures associated with preparing dilution medium by hand and reusing potentially hazardous glass bottles to dilute samples, BBL Disposable Dilution Bottles offer are extremely streamlined, efficient protocol. The laboratorian merely unscrews the lid and peels off the seal; the bottle is ready for use.

BBL Disposable Dilution Bottles come packaged in units of fifty bottles, and are available from any Becton Dickinson Microbiology Systems distributor. Order Peptone Dilution Water, 90 ml fill, catalog no. 4312415; Peptone Dilution Water, 99 ml fill, catalog no. 4312416; Butterfield Buffer (Phosphate Buffered Dilution Water), 90 ml fill, catalog no. 4312420; and Butterfield Buffer (Phosphate Buffered Dilution Water), 99 ml fill, catalog no. 4312421.

Becton Dickinson - Cockeysville, MD

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Affiliate News

The Ontario Food Protection Association 1993 Annual Meeting

Total Quality Confusion — What Does It All Mean?

TQM, ISO 9000, HACCP . . . What is the right management approach today? Where are these quality assurance strategies taking us? The speakers at this year's OFPA Annual Meeting will provide some of the answers.

Wednesday, November 17, 1993
Valhalla Inn, Etobicoke Ontario
Registration: $75 OFPA Members
$95 non-members
Luncheon included

Registration materials were mailed to OFPA members in the September newsletter. For non-members, or for further information, please contact Debbie Labelle, J.M. Schneider, Inc., (519)885-8741 or Anna Lammerding, Agriculture Canada, (519)822-3300.

Upcoming IAMFES Affiliate Meetings

NOVEMBER

•**2-3, North Dakota Environmental Health Association's Annual Meeting** to be held at the Doublewood in Bismarck, ND. For more information, contact Garry Hoffman, ND Department of Agriculture at (701)224-4763.

•**10, Tennessee Association of Milk, Water and Food Protection's Fall Meeting** will be held at the Ellington Agricultural Center Auditorium, Nashville, TN. For more information contact Dennis Lampley at (615)360-0157

•**10-11, Alabama Association of Milk, Food and Environmental Sanitarians** will hold their Annual Meeting at the Howard Johnsons, Montgomery. For more information contact Dr. Tom McCaskey at (205)844-1518.

•**15-17, Pennsylvania Association of Dairy Sanitarians and Dairy Laboratory Analysts Fall Meeting** will be held at Penn State University, University Park, PA. For more information, contact Mike John at (717)762-7789.

•**17, Ontario Food Protection Association Annual Meeting** will be held at the Valhalla Inn, Etobicoke, Ontario, Canada. For more information call Debbie Labelle at (519)885-8741 or Anna Lammerding at (519)822-3300.

IAMFES' Newest Affiliate

The following is a brief historical perspective on MADFES (Metropolitan Association for Dairy, Food and Enviromental Specialists) submitted by MADFES Delegate Fred Weber. The Association was organized in 1936, and according to Maurice Weber, a past president, its main focus has always been the dissemination of all kinds of information for the dairy industry: technical, scientific, production, processing. Our regular monthly meetings, with a speaker, have historically allowed face-to-face interaction on pertinent subjects and concerns on a timely basis.

For example, several recent monthly presentations: "Theory and Application of a Rapid Hygiene Monitoring System", "IMS Requirements on Drug Residue Sampling, Analysis and Reporting" and "Applications of Ultraviolet Disinfection Systems in Dairy Processing". We are currently planning, in addition to our regular meetings, a half to full day seminar for Spring 1994.

The core constituency is from New Jersey and New York. The society has been an affiliate of the New York Association of Milk and Food Sanitarians for many years, however our meetings are held in New Jersey and our long time executive secretary is Professor Dick H. Kleyn of Rutgers/Cook College in New Jersey.

The Society's "best" activity this past year has been it's efforts to become a full IAMFES affiliate. Our decision to apply has several key reasons. Because of the ongoing change in the dairy industry and its demographics, the Society has been experiencing a decline in membership. We recognize IAMFES as a vibrant and growing organization, with a traditional strong emphasis on dairy, and an affiliation will expand the society's base significantly to include foods, water and the environment. In addition, a number of our members are already IAMFES (but non-affiliated) members. This affiliation will highlight the IAMFES agenda on a regular basis to those individuals.

Non-affiliated IAMFES members, who are not Society members, as well as IFT members in our region, will all be contacted. We will also be promoting "corporate" type memberships.

Our president is Don Hammer, 1st V.P. is Eileen Wachowski and Sergeant-at-Arms is Gloria Harman.

New York State Association of Milk and Food Sanitarians Annual Report

This report is based on our year ending August 31, 1992.

New York State Association of Milk and Food Sanitarians has 468 regular members, 22 student members, 84 honorary life members, and 62 sustaining members for a total of 636 members. Which is a drop of about 5% from last year. The tight economic times has had an effect on our sustaining members but we will be working to increase those numbers for 1993.

The Executive Board met five times during the year to guide the organization and to handle the $35,000 budget.

We publish 4 Newsletters plus an Annual Report each year.

The Association holds their 3 day annual meeting in September and also holds a 2 day planning meeting for the annual meeting in April. We had over 275 registrants for

the 1992 meeting. The attendance was up by approximately 15% over 1991. This is getting closer to our normal attendance. The Association has a Farm Methods Committee, a Food Committee, a Laboratory Committee, and a Dairy and Food Equipment Committee, each of which meets at least twice a year.

There are 14 affiliates of the State Association. Bill Young is the Council of Affiliates Chairman and has a minimum of two meetings of the Affiliate Delegates each year. The affiliates each hold from four to seven meetings annually. Each year the George "Sid" Miller Affiliate of the year award is presented to one of the affiliates. Several different criteria are used to make the selection including activity in the state organization.

After this meeting we will have 13 affiliates because one of our affiliates just became an IAMFES affiliate from New Jersey.

The association presents 5 major awards each year during their awards banquet.

The association is a member of the Dairy Recognition & Education Foundation. We have a Delegate attend the annual meetings and contributions are made by our association to the foundation in memory of our departed members. All State association educational and scholarship activities are through the Foundation. Some of the local affiliates have scholarship programs.

The 1993 Annual Meeting is scheduled for September 20-22, 1993 at the Holiday Inn, Genesee Plaza, Rochester, NY. We have an excellent program planned and we would welcome your attendance.

Terry B. Musson
Delegate NYSAMFS

Abstracts of Papers Presented at the 80th Annual Meeting of IAMFES

Atlanta, Georgia — August 1-4, 1993

Abstracts of most papers submitted for presentation at the 80th 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.*

IVAN PARKIN LECTURE

THE CHALLENGES OF EPIDEMIOLOGY IN FOOD PROTECTION

Morris E. Potter, Assistant Director for Bacterial and Mycotic Diseases at the Centers for Disease Control and Prevention, 1600 Clifton Road, NE, Atlanta, GA 30333

Epidemiologists have played an important role in food safety in the past by identifying foodborne hazards and trends in the occurrence of foodborne illnesses. Changes in foods, in microorganisms, and in consumers will create opportunities for new food safety problems, so epidemiologists must continue to scan the horizon for emerging foodborne diseases. However, the challenges of epidemiology relating to food protection as we move toward the third millennium will require refining and redirecting the traditional roles of epidemiologists. For epidemiologic data to establish public and professional food safety priorities by product, process, and population, the data must be stronger and communication about risk must be improved. In addition, greater effort to effectively merge epidemiologic and microbiologic data will be necessary to fully characterize foodborne risk.

NEW HORIZONS IN DAIRY FOOD SAFETY AND QUALITY

MICROBIAL INDICATORS FOR DAIRY FOOD PROCESSING

Peggy M. Foegeding*, Associate Professor, and T. M. Fairchild, North Carolina State University, Box 7624, Raleigh, NC 27695-7624

Many industries use biological indicator organisms (BI) to evaluate the lethality of a process. A BI is added to a test batch of the pre-processed product, the product is processed, and the survival of the BI is evaluated to assess the process lethality. In the food industry, a classic example is the use of *Clostridium sporogenes* (PA3679) spores to evaluate canned food processes for inactivation of *C. botulinum.* The use of a properly selected BI is a positive, proactive approach to verify whether a process provides the requisite or desired level of safety. The use of *Listeria innocua* ATCC 33091 as a BI for monitoring mild thermal processes (pasteurization) for inactivation of *L. monocytogenes* has been proposed. Use of this BI has been simplified by marking it with antibiotic resistance using electrotransformation or natural mutation. This permits easy, quantitative recovery when the population of the BI is a minority of the total microbial population in the food. These indicators have been applied successfully in several academic and industrial laboratories for evaluation of thermal processes of milk, egg, and meat products. Similarly, BIs could be identified for other processing objectives, such as extended shelf-life refrigerated foods. Guidelines for selection of an appropriate BI and limitation to their use will be discussed.

PREDICTIVE METHODOLOGIES TO RAPIDLY ASSESS SHELF-LIFE

Charles H. White, Food Science and Technology Department, Mississippi State University, Mississippi State, MS 39742

The need for dairy processors to rapidly predict the shelf-life of their products is becoming even more critical. The tests that are available to the quality assurance personnel are many. The requirements of each of these tests help to determine which tests would be appropriate in each given situation. Tests involving general plating procedures coupled with preliminary incubation (PI) will be discussed as will methods designed to rapidly detect and quantify bacterial metabolites as they are produced under the test conditions. These methods will include impedance microbiology, bioluminescence, reflectance colorimetry, and catalase measurement. Emphasis will be given to the current status and availability of each of these procedures. Further, how predictive testing fits into an overall quality assurance program will also be mentioned.

RAPID IMMUNOLOGICAL AND DNA TECHNOLOGIES TO DETECT MICROBIAL PATHOGENS

Mike Johnson*, Professor, A. K. Bhunia, R. F. Wang, W.W. Cao and P. J. Steele, University of Arkansas, Department of Food Science, 272 Young Avenue, Fayetteville, AR 72703

Many methods claimed to be rapid are not when the initial isolation time is added. Our lab has focused on mouse hybridoma cells (MHC) producing monoclonal antibodies (MAb) and on polymerase chain reaction (PCR) assays based on unique 16s-rRNA base sequence probes that will specifically detect *Listeria monocytogenes* (*Lm*) in less than 24 h total. The MAb probe has been incorporated into a microcolony-immunoblot assay that permits direct enumeration of as few as 6 CFU of *Lm*/g of food in 24 h. This MAb is currently being evaluated by several kit companies. Another MAb specific for *Pediococcus acidilactici* has lead to isolation of a new pediocin, RS2, with an amino acid sequence different from that of AcH. PCR assays to detect as few as 4-40 cells of *Lm* or of *L. ivanovii* required only 3 h with microcentrifuge tubes or 45 min with capillary tubes plus 1 h electrophoresis to separate the PCR products and required no additional time for DNA isolation or hybridization. An in vitro MHC assay was able to detect virulent *Lm* strains in 4-6 h vs 3 days for immunocompromised mice. Rapid assays form other laboratories for *Lm, E. coli* O157:H7, *Vibrio* spp. and other pathogens will be reviewed briefly. [Supported by grants from USDA-NRI, Food Safety Consortium, and SEPEA].

ANTIMICROBIAL PROTEINS FOR DAIRY FOOD SYSTEMS

Todd R. Klaenhammer, Director, Southeast Dairy Foods Research Center, North Carolina State University, Raleigh, NC 27695-7624

The lactic acid bacteria, as well as endproducts of their growth and metabolism, are "generally recognized as safe" due to their long association with mankind and his food supply. These bacteria also produce a variety of antimicrobial compounds that inhibit or kill pathogenic bacteria. Foremost among these are antimicrobial proteins, termed bacteriocins, which have become increasingly important as natural food preservatives. In the search for new and improved food preservatives, bacteriocins produced by the lactic acid bacteria offer many new opportunities to design natural and more effective antimicrobial systems. This presentation will discuss the different classes of bacteriocins, their mechanisms of action, and illustrate practical examples of how bacteriocins may be used in the dairy and food industries to extend the margin of safety.

TECHNICAL SESSION — DAIRY

INHIBITION OF GRAM-POSITIVE PATHOGENS IN COLD-PACK CHEESE MADE FROM CHEESE CONTAINING NISIN

T.L. Yezzi*, Research Assistant, A.B. Ajao & E.A. Zottola, Department of Food Science and Nutrition, University of Minnesota, St. Paul, MN 55108

Research has shown that the incorporation of nisin into food products inhibits gram (+) pathogenic microorganisms. In research reported earlier, our laboratory was able to manufacture Cheddar Cheese with up to 1200 IU nisin per gram of cheese.

Cold-pack cheese was manufactured using the nisin containing cheese. Cold-pack cheese made this way was inoculated with *L. monocytogenes* V7, *S. aureus* 196E or *C. sporogenes* PA3679 spores. Fifty gram samples were sealed in plastic pouches. Samples inoculated with *L. monocytogenes* V7 were stored at 4, 7, and 23°C. Samples containing *S. aureus* 196E cells and *C. sporogenes* PA3679 spores were stored at 23 and 37°C. The cold-pack

cheese samples were examined for numbers of viable bacteria or spores at 0, 3, 7, 14, 21, 28 and 56 days.

All samples showed some reduction in numbers of microbes over time. A greater reduction in the total number of microorganisms was observed as nisin concentration and temperature was increased. Numbers of *Clostridium* spores and *S. aureus* cells were reduced from 1000 to <3 and <10 per gram respectively in 28 days. *L. monocytogenes* was reduced from 2,000 to 10-100 in 21 days. After 21 days the number of *L. monocytogenes* persisted at levels of 10-100 cfu/g cheese. It appears that using nisin-containing cheese as an ingredient in cold-pack cheese is effective in controlling these gram (+) pathogens.

ANTIMICROBIAL USE AND DAIRY DISEASE PATTERNS

Rick Bennett, Ph.D., Water and Food Science Advisor, University of California, Cooperative Extension, 2604 Ventura Avenue, Room 100, Santa Rosa, CA 95403

Antimicrobial residues in the commercial milk supplies is a major concern within the dairy industry. Clinical bacterial mastitis is the most common disease on the dairy farm and treatment is the probable source of violative antimicrobial residues in commercial milk. The purpose of this study is to evaluate the patterns of clinical mastitis and antimicrobial use on a large western dairy farm during a 24 year transition from mastitis caused by contagious pathogens *S. aureus* and *St. agalactiae* to mastitis caused exclusively by environmental pathogens (Coliforms and environmental streptococci).

Clinical mastitis records from a 350 cow herd for the period 1966 to 1987 were evaluated. The number of cases and the number of antimicrobial treatments per case were determined. The data was analyzed on a month by month basis over the 24 year period.

For the period of predominantly contagious mastitis (1966-1974), the number of cows treated for clinical mastitis ranged from 141 to 761 cases per year. In the period from 1979 to 1989 environmental mastitis predominated. The number of cases and case rates ranged from 128 to 215 and .41 to .71 respectively.

The number of days of treatment and the total number of treatments were significantly correlated (r=.97) only for the contagious mastitis period 1966 through 1974. The total number of treatments for that period was 1,659, versus 951 for the period of environmental mastitis, 1975 - 1989.

The use of antimicrobials on the dairy is closely associated with the total number of clinical cases. There appears to be greater drug use with the contagious pathogens. This case study suggests antimicrobial use in dairy cattle was more common during the periods of active contagious mastitis infection. The reduction of contagious mastitis at the national level should be associated with decreased antimicrobial use and incidental residues.

A RAPID DIPSTICK BIOSENSOR FOR BETA-LACTAMS IN MILK

Richard M. Rocco*, Ph.D., Vice-President and Director of Research, S.S. Deshpande, S.V. Kharadia, and L.S. Jang, Idetek, Inc., 1245 Reamwood Avenue, Sunnyvale, CA 94089

A new rapid biosensor based immunoassay has been developed for measuring residual beta-lactam (BL) drugs in fluid dairy products including raw milk. Total test time is under five minutes and consists of adding a measured amount of milk to a single stable reagent followed by a short room temperature incubation with the Biochip biosensor. The binding of BL drugs to specific antibodies causes proportional changes in the optical surface characteristics of the disposable 4x5 mm biosensor. The Biochips are read in a small dedicated bench-top reader. Several Biochips may be mounted on the same plastic holder for simultaneous multianalyte testing in a single sample of milk. Specificity for the drugs of interest is achieved through the use of direct monitoring of antibody binding without the use of enzyme conjugates or secondary color reactions. Detection limits for the various BL drugs is less than 3 ppb. Between run precision CV's for cephapirin and ampicillin are typically under 10% at 20 and 5 ppb respectively. No interferences were observed in finished diary products with fat contents of up to 40% or in individual and bulk tank samples from infected but non-treated cows.

USE OF THE PIG AS A MODEL TO STUDY COLONIZATION OF THE GASTROINTESTINAL TRACT BY BIFIDOBACTERIA AND *LACTOBACILLUS ACIDOPHILUS*

D. H. Toop*, Graduate Student, C. L. Duitschaever, C. Buteau, C. L. Gyles, and B. Allen, University of Guelph, Department of Food Science, Guelph, Ontario, Canada N1G 2W1

Lactobacilli and bifidobacteria constitute the predominant microflora in a healthy intestinal environment. A shift in the balance accompanied by the disappearance of bifidobacteria usually results in a loss or weakening of protection against infection. Reequilibration of the intestinal microflora by feeding lactobacilli and bifidobacteria in the form of a fermented dairy product requires that large numbers of viable microorganisms adhere to the epithelial surface of the intestinal tract. A single feeding of bifidobacterium and *L. acidophilus* was made to pigs using a fermented dairy product.

Antibiotic resistant marked strains of bifidobacteria and *L. acidophilus* were used to follow colonization of these bacteria in the gastrointestinal tract of the pig. Adherence to intestinal epithelial cells was also determined. Both organisms were recovered from the gastrointestinal tract several days after a single feeding of a fermented dairy product.

PROBLEM SOLVING IN A DAIRY QUALITY CONTROL LABORATORY USING A PC DATABASE

David F. Blomquist*, Laboratory Information Management Specialist, and R. L. Bakka, Klenzade, a Service of Ecolab, 11222 Bloomington Drive, Tampa, FL 33635

A growing number of dairy laboratories have installed computer databases to monitor the quality of their raw materials and products. The computer is proving to be a very effective tool in monitoring control points in dairy processing. The applications of computer databases in several plants has shown that this relatively new tool is valuable for QC applications for controlling and improving quality of dairy products.. Introduction of a database will cause considerable change in a QC laboratory. Instead of handwritten data sheets, a Quality Manager evaluates summaries of specific products, lines, times of the day or other variations from computer printouts which are quickly generated. With improved data management, problem resolution is possible at speeds that were not previously possible.

REGULATORY ISSUES OF BIOTECHNOLOGY

REGULATION OF BIOTECHNOLOGY-DERIVED FOODS

Eric Flamm, Office of Biotechnology, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857

I will discuss FDA's policy on foods derived from new plant varieties developed using biotechnology. I will describe the kinds of newly introduced substances that would need FDA approval and the kinds that would not; the kinds of special labeling issues that can arise; and the kinds of general safety questions that should be answered, such as level of significant nutrients, natural toxicants, and potential for altering the allergenicity of the food. And I will discuss some of the comments we have received about our policy and our plans to address the comments.

THE BST APPROVAL PROCESS

Stephen F. Sundlof, D.V.M., Ph.D., University of Florida, College of Veterinary Medicine, P.O. Box 110925, Gainesville, FL 32611-0925

Recombinant bovine somatotropins (rBST) are effective drugs for increasing milk production in dairy cows. FDA approval of rBST is actively being pursued by 4 prominent pharmaceutical firms at unprecedented costs. One firm alone has submitted approximately 200,000 pages of data and information to FDA resulting in a review process now exceeding 10 years. By historical standards, this represents the largest submission for any new animal drug. In addition to the FDA review, congressional oversight and various expert panels have scrutinized, not only the data, but FDA's interpretation of the date and the agency's compliance with regulations governing the drug approval process. This presentation will focus on the findings of these panels and on the unique socioeconomic forces impeding the regulatory process.

LISTERIA MONOCYTOGENES: CURRENT ISSUES AND CONCERNS SYMPOSIUM

Sponsored by the International Life Sciences Institute

LISTERIA MONOCYTOGENES: STATE OF THE SCIENCE

Jocelyne Rocourt, Institut Pasteur, Laboratoire des *Listeria*, 28, rue du Dr. Roux, 75724 Paris CEDEX 15, France

In 1992, a major outbreak of listeriosis was observed in France which included 279 cases from 79 of the 96 departments in metropolitan France over a 9-month period. Extensive epidemiological investigations, including a case-control study as well as microbiological analysis of food samples from

various origins, revealed a strong association (odds ratio: 9.2; confidence interval: 3.8-22.4) between consumption of pork tongue in aspic and infection in 47% of patients. Analysis of results gathered during this epidemic led to a number of conclusions:

- A national surveillance system, based on strain typing, was essential; without it, this outbreak would not have been detected because of the low attack rate and the wide geographical distribution. The system is not exhaustive, but is sensitive enough to detect an unusual phenomena.
- The detection of the responsible food was complex since it was nationally distributed; microbiological investigation of foods found in patient refrigerators was hampered by the duration of incubation (from a few days to a few weeks) and by numerous cross-contaminations. The investigation of food in retail stores frequented by patients was far more informative.
- Most epidemic patients belonged to populations at risk; pregnant women and neonates (33%), and adults with underlying disease (61%). A transient immunologic defect, which could explain why listeriosis occurred in some apparently "healthy" individuals, was not documented.
- As was the case in previous outbreaks, the major vehicle was a food at risk, which easily sustains multiplication of *L. monocytogenes*.
- Results of case control analysis and enumeration of *Listeria* in the pork tongue and in other contaminated meat products suggested a dose-effect relationship.
- Due to its capacity to grow at 4°C and to its widespread occurrence in the environment, *Listeria* has become part of the microbial ecosystem of food production and processing, making identification of the origin of food contamination especially difficult.
- At least three different typing methods were required to precisely identify food contaminated with the epidemic strain. More than 15,000 strains were screened using serogrouping and phage-typing; strains belonging to the epidemic phagovar were further characterized by DNA macrorestriction patterns. The latter step revealed strong heterogeneity within the strains isolated from foods.
- Preliminary studies indicated that the epidemic strain belonged to a group of strains highly virulent toward mice.
- Why strains of the phagovar, responsible for only 2 to 9% of sporadic cases a few years ago in France, should suddenly emerge in 1992 as an epidemic strain remains unexplained. In addition, as concerns the typing methods used, this strain shares high phenotypic and genomic relatedness with strains responsible for the California and Swiss outbreaks.

INDUSTRY PERSPECTIVES ON *LISTERIA MONOCYTOGENES* IN FOODS: RAW MEAT AND POULTRY

James Marsden, Vice President, Science and Technology, American Meat Institute, P. O. Box 3556, Washington, DC 20007

Industry is committed to reducing the incidence of *Listeria monocytogenes* in both raw and cooked meat and poultry products. Pathogenic organisms are often present in small numbers and are part of the natural microbial flora of live animals. *Listeria monocytogenes* acts as an opportunistic pathogen which has the capacity to sustain itself in humans. Because of its nature, *Listeria monocytogenes* can occasionally cause disease in compromised individuals. Thus, infection and disease are as dependent on the host as on the microorganism. This is the reason that the infectious dose is dependent on the virulence of the organism and the obesity of the host immune system to resist infection.

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 *Listeria monocytogenes* and other pathogens in raw product. New technology developed in part by AMI research on organic acid carcass sprays and decontamination procedures offers practical means to reduce microbial contaminants. Research is also being conducted to determine the effect of low dose gamma irradiation on *Listeria monocytogenes* and other pathogens in ground beef. Preliminary results indicate that this technology is effective. Through the application of thermal processes that assure the destruction of *Listeria monocytogenes* and through the wide use of HACCP and GMP's, improved sanitation, separation of raw materials from cooked product and other measures designed to reduce the probability of recontamination, the incidence of *Listeria monocytogenes* in cooked ready-to-eat produce is exceedingly low. However, unless these products are absolutely sterilized, the incidence is not likely to ever by zero; and even if the incidence is zero, recontamination and outgrowth may occur further down the food chain.

The absolute elimination of *Listeria monocytogenes* from the food supply is not a realistic objective. Additional measures can be taken to prevention Listeriosis. An educational program should be developed which would be targeted at physicians and at risk individuals on food choice decisions and appropriate preparation methods to assure *Listeria monocytogenes* is not present in the food they eat. Research is needed to gain a better understanding of the pathogenicity of *Listeria monocytogenes* in humans and a better methodology is needed to differentiate virulent from nonvirulent strains and to identify genetic markers associated with invasiveness.

INDUSTRY PERSPECTIVES ON *LISTERIA MONOCYTOGENES* IN FOODS: MANUFACTURING AND PROCESSING

Dane Bernard, National Food Processors Association, 1401 New York Avenue, N.W., #400, Washington, DC 20005

While there is not universal agreement of, nor acceptance of a zero tolerance for *Listeria monocytogenes* for all ready-to-eat foods, many segments of the food industry have developed control and intervention strategies to achieve a reduction in possible presence of *Listeria monocytogenes* in foods. The Hazard Analysis Critical Control Points (HACCP) concept has been utilized to systematically discern what critical control points (CCPs) could be identified and what control procedures can be instituted. In products which may be packaged and handled without benefit of a pasteurization or other inactivation step, these control measures have focused mainly on sourcing of ingredients which are *Listeria*-free, treatments of ingredients to render them *Listeria*-free, and an emphasis on targeted sanitation and sanitary practices in the plant. For products which may incorporate a pasteurization or kill step, efforts have focused on assuring that the kill step is adequate and on avoidance of recontamination after application of the kill step. Specific procedures and techniques used in these efforts will be discussed along with the overall need to redress regulatory policy regarding *Listeria monocytogenes* in ready-to-eat foods.

INDUSTRY PERSPECTIVES ON *LISTERIA MONOCYTOGENES* IN FOODS: RETAIL DISTRIBUTION

Catherine Adams, Director, Scientific Affairs, Grocery Manufacturers of America, 1010 Wisconsin Avenue, N.W., Washington, DC 20007

According to data from the Centers for Disease Control, the incidence of listeriosis in the United States has declined by 40 percent between 1989 and 1992. This decline is attributable at least in large part to enhanced awareness and improved control of *Listeria monocytogenes* by the food industry, including the food manufacturer and the retailer. HACCP controls have provided the key to risk management by the industry. However, it is clear that the presence of the microorganism cannot be completely eliminated. The answer is for optimal control of *Listeria* in foods through retail distribution and storage until the point of consumption. Regulatory agencies should depend in the future on risk management decision making, given evidence of pathogen control. The application of HACCP programs by the industry will provide the evidence and track record of *Listeria* control. Education programs must continue for the industry, including the retail sector, regarding the proper design and application of HACCP programs. The food safety initiatives of the regulatory agencies may be helpful in institutionalizing effective HACCP programs across the industry, if applied appropriately and are not overly prescriptive.

REGULATORY CONCERNS OF THE U.S. DEPARTMENT OF AGRICULTURE

Ann Marie McNamara, Director, Microbiology Division, Food Safety and Inspection Service, U.S. Department of Agriculture, 300 12th Street, S.W., Room 410 Annex, Washington, DC 20250

In three separate sessions, the USDA perspective on three foodborne pathogens will be presented. Included in the presentations will be current data from the Food Safety and Inspection Services' testing program for *Listeria monocytogenes* in cooked, ready-to-eat meat and poultry products, data on the recovery of *L. monocytogenes, E. coli* O157:H7, and *Campylobacter jejuni* from raw product surveys, current USDA methods for identification of these bacteria including results of USDA methods development work, and a brief overview of USDA's participation in the recent *E. coli* O157:H7 outbreak in Washington State.

REGULATORY CONCERNS OF THE U.S. FOOD AND DRUG ADMINISTRATION

Joseph M. Madden, Director, Division of Microbiology, Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, 200 C Street, S.E., Washington, DC 20204

Three human foodborne pathogenic bacteria have dominated the food safety literature for the past 10-15 years: *Listeria monocytogenes, Escherichia coli* O157:H7, and *Campylobacter jejuni*, and all have been found in food products regulated by the FDA.

Listeria monocytogenes has been responsible for the deadliest foodborne outbreak ever recorded in U.S. history, accounting for forty-eight deaths among one hundred forty-two individuals infected with the microbe in 1985. This microbe is widely distributed in nature, and has been isolated from fresh vegetables, milk, cheese, fruits, meats, and seafood. It is unusual in its ability to reproduce at refrigerator temperatures, and some evidence suggests that the ingestion of small numbers of viable microbes may result in illness. The incidence of listeriosis in the U.S. is declining from highs of 8.9 cases/100,000 neonates and 18 cases/100,000 adults in 1986 to 4.0/100,000 neonates and 8.2/100,000 adults in 1992. Regulatory initiatives and educational campaigns have been credited with this reduction.

EPIDEMIOLOGY OF LISTERIOSIS IN THE UNITED STATES

Anne Schuchat, Medical Epidemiologist, Division of Bacterial and Mycotic Diseases, National Center for Infectious Disease, National Centers for Disease Control, Mailstop C09, Atlanta, GA 30333

Since the 1985 outbreak of listeriosis in Los Angeles, efforts to characterize the epidemiology of listeriosis in the United States focused on determining the incidence of and identifying risk factors for sporadic disease due to *Listeria monocytogenes*. Data from multistate laboratory-based active surveillance in aggregate populations of 18 to 34 million are used to make national projections of disease burden. In conjunction with active surveillance for invasive listeriosis, periodic laboratory audits are conducted which demonstrate that the system is over 95% sensitive. The incidence of listeriosis was similar in 1986 and 1989, but recent data suggest that the incidence decreased during 1990 through 1992, coinciding with substantial efforts taken by the food industry and regulatory agencies to reduce contamination by *Listeria* and prevent sale of any ready-to-eat processed foods in which *L. monocytogenes* was detected. Decreases occurred in the incidence of perinatal listeriosis as well as disease in persons >50 years old. Although natural variation may account for these changes, the reduction in cases and deaths due to *L. monocytogenes* may reflect improvements in the safety of the food supply or changes in dietary practices of persons at risk. Surveillance data suggests that prevention efforts may be having an important effect on the incidence of listeriosis, and provide justification for continuation of these efforts. Ongoing surveillance for listeriosis will be important to monitor these encouraging trends.

EUROPEAN PERSPECTIVES ON *LISTERIA MONOCYTOGENES*

Paul Teufel, Institute for Veterinary Medicine, BGA, Diedersdorfer, Weg 1, W-1000 Berlin 48

Three major outbreaks of Listeriosis in Europe have confirmed an unmistakable connection between contaminated food and illness. The detection of *Listeria monocytogenes* in a number of foods such as poultry, meat and meat products, fish and fish products, prawn and shrimp meat as well as in pre-shredded mixed salads caused major problems for the food surveillance authorities with respect to the legal classification and actions to be taken.

The application of a zero tolerance to all foods would lead to an unjustified rejection of many foods, particularly raw foods. However, for certain foods, zero tolerance can be applied. A strategy that can be supported by both the producers and the surveillance authorities should aim to prevent contamination by *Listeria* as far as possible or to subject apparently short-term contamination of some foods to a standardized form of assessment.

The minimal infective dose of *L. monocytogenes* needed to cause infection in humans is not known. Given the wide prevalence of *Listeria* in both the living and inanimate environment and the low incidence of infection is reason to assume that a health risk exists, particularly in the presence of a high bacterial count. Consequently, it seems necessary to enumerate the *Listeria* contamination of food. Quantitative assessment, type of food, and the ability of national legislatures to implement appropriate actions, should form the basis for a balanced approach to the evaluation of *L. monocytogenes* in foods.

For foods in which *Listeria* cannot be completely avoided, a general microbiological limit of less than 100 *L. monocytogenes* per gram at the point of sale to the consumer is used in several European countries (mostly as recommendation). Values above 100 (e.g. in Germany) are not additional tolerance values but determine the type of action to be taken by food inspection authorities. EEC has only regulated *L. monocytogenes* in cheeses at the end of manufacture (absence in 25 g). When *L. monocytogenes* contamination is evaluated, no differences are made between different serovars or epidemic strains. In addition to these recommendations for food inspection services, educational activities, particularly for pregnant women and immunocompromised people, are issued by national health authorities.

A quantitative evaluation of approximately 15,000 different food samples in Germany revealed that (i) since 1986 the number of positive samples had decreased; (ii) only very few food items of certain producers had high numbers of *Listeria*, e.g. vacuum-packed, sliced sausages and vacuum-packed trout fillets, (iii) in case of positive results, a sequential sampling plan (n=5) helps food inspection authorities to focus on products under suspicion; (iv) the quantitative approach to regulating *L. monocytogenes* contamination of foods is feasible and effective.

STATUS OF *LISTERIA MONOCYTOGENES* IN THE CANADIAN FOOD INDUSTRY

Anna M. Lammerding*, and J.M. Farber, Section Head of the Food Safety/New Hazards Laboratory, Health of Animals Laboratory, Agriculture Canada, 110 Stone Road, West, Guelph, Ontario, Canada N1G 3W4

As our understanding of the epidemiology of *Listeria monocytogenes* improves, it is becoming clear that if fundamental principles of food safety are diligently applied at all levels of the food chain, the risk of foodborne disease caused by *L. monocytogenes* can be minimized. Canadian regulatory agencies have implemented a control strategy for foodborne listeriosis which takes into account that the total elimination of *L. monocytogenes* from all foods may be impractical and impossible to achieve. Rather than focusing on end-product testing, in-depth environmental sampling is conducted in food processing plants to identify potential points where recontamination of ready-to-eat products can occur. A three-phase approach is used to ensure adherence to good manufacturing practices (GMP's), and compliance with the Food and Drug Act. This approach stresses overall plant sanitation and management strategies which incorporate hazard analysis and critical control point (HACCP) techniques. A federal laboratory accreditation program (Agr. Can.) exists for laboratories which demonstrate a high standard of quality in procedures to isolate *L. monocytogenes* from meat products and environmental samples. About 45 to 60 cases of sporadic listeriosis are reported each year in Canada; however, no foodborne outbreaks have been reported since 1981. By continually reviewing and improving GMPs and conducting frequent inspections in food plants, Canadian food processors and regulators are striving to minimize the risk of foodborne listeriosis.

LISTERIA MONOCYTOGENES AND FOOD: THE UK APPROACH

Diane Roberts, Deputy Director, Public Health Laboratory Service, Food Hygiene Laboratory, Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT United Kingdom

In the late 1980's the United Kingdom saw a sharp increase in the number of reports of cases of human listeriosis and also of the presence of *Listeria monocytogenes* in a wide variety of foods. Public concern was raised by some rather "hysterical" items in the media which required action to be taken by the Government. Three areas will be examined: a) the recent pattern of human listeriosis in the UK b) the occurrence of *L. monocytogenes* in foods and c) the response by Government and the food industry.

Laboratory reports in the UK show a steady increase in the number of cases reported between 1983 and 1988 from 131 to a peak of 327 with a doubling of cases between 1986 and 1987. From mid 1989 to 1990 and 1991 there was a dramatic decline to 131 (1990) and 143 (1991) cases and reports for 1992 remain at this low level. Much of the increase between 1987 and mid 1989 was due to two subtypes of *L. monocytogenes*, serotype 4b phage type 6,7 and serotype 4bX. Contaminated imported pâté was identified as the most likely source of these strains. The decline in listeriosis from mid 1989 coincided with Government Health warnings to vulnerable groups concerning the consumption of pâté and removal of the product of one manufacturer from the market.

Tests for the presence of *L. monocytogenes* in foods are now carried out by most food microbiology laboratories in the UK as part of planned surveys or routine surveillance studies. Methods of isolation have been greatly improved and refined and results from the PHLS Food Microbiology External Quality Assessment Scheme indicate that performance with the isolation of *L. monocytogenes* is better. The presence of the organism has been reported in a wide variety of food types examined except those which receive a stringent heat treatment within their final packaging (e.g. canned foods).

The response of the Food Industry to adverse publicity about *L. monocytogenes* in their products and the occasional recalls of contaminated foods has been to look closely at their own food production and control procedures. Hazard Analysis Critical Control Point procedures are being more widely introduced as well as voluntary Codes of Practice and greater numbers of tests on product and factory environment are being carried out.

The response by the UK Government was not to introduce legislation requiring the absence of *L. monocytogenes* in specific products - although it is required to incorporate into home food law the microbiological standards included in EC Directives. Rather it has sought to establish the facts, then provide clear advice to vulnerable groups, initiate research and introduce new legislation which will help control the multiplication of the organism. The

CALL FOR PAPERS

IAMFES 81th Annual Meeting

July 31 - August 3, 1994
San Antonio, Texas

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, 1993 to:

Steven K. Halstead, CAE
Executive Manager, IAMFES
200W Merle Hay Centre
6200 Aurora Avenue
Des Moines, IA 50322

Enclose *two* stamped, self-addressed post cards. 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 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.

Ten finalists will be selected for the Poster Competition. The presentation must be mounted on a 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 page 557 of *Dairy, Food and Environmental Sanitation* September Issue and the following blue pages.)**

All winners are presented and honored at the annual Awards Banquet. The ten 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 photo copy this one.

Membership in IAMFES

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

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Please type abstract, double-spaced, in the space provided here.

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Judging Criteria for Developing Scientist Awards Competitions

Judging

The abstracts and presentations will be evaluated by an independent panel of judges. Selection of ten 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, 1994.

Only the ten finalists in each category will be judged upon their final presentations 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.

Judging Criteria

ABSTRACTS

Short abstract: clarity, comprehensiveness, conciseness;
Extended abstract: technical merit, organization, completeness;

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: Both a short abstract and an extended abstract must be submitted to the IAMFES office no later than December 15, 1993. No forms will be sent to entrants. Enclose two self-addressed, stamped postcards with your submitted abstracts.**

Instructions for Preparation of Extended Abstract:
Type your abstract, single-spaced, using elite (12 pitch) letter-quality type, on 8.5" x 11" pages. The margins should be as follows: Top: 1"; Bottom: 0.75"; Left: 1"; Right: 1". Do not exceed 3 pages, and DO NOT attach additional tables or graphs.

A. The first section should occupy the first fifth of the first page and read as follows:

First 3 lines or less, type:
TITLE: Capitalize only the first letter of the title and first letters of proper nouns.

Leave a blank line, then in the next 2 lines or less, type:
AUTHORS: Capitalize name of SPEAKER ONLY.

Leave a blank line then in the next 4 lines or less, type:
AFFILIATIONS: Name and complete mailing address of Affiliation.

Leave a blank line then on the next line, type:
Developing Scientist Awards Competition: Oral or Poster.

Leave a blank line then type:
Professor (or Department Head): Have your Professor or Department Head sign here.

B. Leave two blank lines then state briefly (8 lines or less):

"OBJECTIVES"
Indent the first line 5 spaces.

Leave a blank line, then describe:
"METHODS"
This should take up a maximum of three-quarters of a page; continue on page 2 if necessary. Include sufficient detail to indicate the adequacy of the experimental design and difficulty of research.

Leave a blank line then describe:
"RESULTS AND DISCUSSION"
This should take up a maximum length equivalent to 1 page; continue on page 3 if necessary. This section should indicate the extent to which objectives were met and validity of conclusions based upon data.

Leave a blank line then describe:
"SIGNIFICANT FINDINGS, CONCLUSIONS AND IMPLICATIONS"
This section should take up a maximum of 15 lines and should indicate the technical merit and contribution to science of the work.

Leave a blank line then list:
"REFERENCES":
List a maximum of four significant references. At the end of this section you will probably be close to the bottom of page 3.

conclusions of the WHO Working Group on Foodborne Listeriosis are seen as eminently sensible - 'The elimination of *L. monocytogenes* from all food is impractical and probably impossible ... the critical issue is not how to prevent its presence but how to control its survival.'

AUSTRALIAN PERSPECTIVES ON *LISTERIA MONOCYTOGENES*

Michael Eyles, Product Manager, CSIRO Food Research Lab, P.O. Box 52 North Ryde, New South Wales 2113, Australia

Listeriosis has been a notifiable infectious disease in most States of Australia since 1991. About 40 cases have been reported nationally per year to April 1993. Listeriosis has been associated with a food vehicle on only two occasions. The implicated foods were pâté and mussels. The circumstances surrounding some other clusters of cases have suggested the existence of an unidentified common source of infection. During the last five years the Australian food industry has supported a substantial amount of research on *L. monocytogenes*. The research has focused on the sources and mechanisms of dissemination of *Listeria* in dairy and meat processing operations, rapid methods for the detection of *Listeria*, epidemiological techniques for tracing *L. monocytogenes* in food processing operations and the community, and the development of predictive models for the growth of *L. monocytogenes*. Where necessary the industry has modified its procedures and quality systems to control hazards associated with *L. monocytogenes*. Because of outbreaks associated with dairy products elsewhere in the world, the dairy industry has been particularly active in the development of appropriate control measures. Its procedures are documented in the nationally-accepted Australian Manual for Control of *Listeria* in the Dairy Industry. The incidence of *L. monocytogenes* in Australian dairy products is extremely low. Control measures for other industries are not as well defined. Health authorities have become involved in consumer education on listeriosis and have published dietary guidelines for at-risk individuals. The guidelines advise susceptible persons to avoid particular foods and recommend that certain food preparation and storage procedures that may increase the risk of listeriosis should be avoided.

TECHNICAL SESSION — ANALYTICAL METHODS

THE VALUE OF A DNA PROBE - HGMF PROCEDURE TO DETECT *SHIGELLA*/ENTEROINVASIVE *E. COLI* AND VTEC IN FOOD

E. C. D. Todd*, J. MacKenzie and C. Munro, Bureau of Microbial Hazards, Health Protection Branch, Sir F.G. Banting Research Center, Ottawa, Ontario, Canada.

An enzyme-linked antibody (ELA) procedure combined with growth of organisms on HGMFs has been successfully used to isolate *E. coli* and *Salmonella* from foods. This approach has been modified to allow specific genes to be detected. Primers for the invasive plasmid gene found in *Shigella* and enteroinvasive *E. coli* (EIEC) were used to prepare a 760 bp PCR product. Libraries of 35 *Shigella* and 5 EIEC strains were grown overnight on nutrient agar, and colonial growth lysed to expose the DNA, which was hybridized with the PCR product labeled with digoxigenin from a commercial kit. Only strains known to have the invasive gene gave a positive reaction. To test the value of this procedure for food microbiology, *Shigella* and other organisms were added to 2% milk and filtered through the HGMFs. Only the *Shigella* were successfully detected on the HGMFs.

For verotoxigenic *E. coli* a 340 bp probe was generated using VT2 primers and incorporated digoxigenin-labeled dUTP directly into the PCR reaction. This probe reacted with all but one of 65 VTEC strains and cross-reacted with only one (*C. freundii*) of 217 non-VTECs. This probe was successful in detecting VTEC in hamburger meat.

DEVELOPMENT OF A SIMPLE REVERSE TRANSCRIPTASE-POLYMERASE CHAIN REACTION METHOD FOR THE DETECTION OF ENTERIC VIRUSES IN OYSTERS

Lee-Ann Jaykus*, Graduate Research Assistant, R. DeLeon, & M.D. Sobsey, Department of Environmental Sciences CB#7400, University of North Carolina, Chapel Hill, NC 27599-7400

Enteric virus transmission due to the consumption of fecally-contaminated shellfish is a significant public health concern. Existing methods of detection are time consuming, tedious, and lack sensitivity. The nucleic acid amplification technique of polymerase chain reaction (PCR) offers an opportunity to enrich a specific nucleic acid sequence up to a million-fold and hence improves the lower limit of detection to one virus unit while retaining exquisite specificity. Our goal was to develop methods to purify and concentrate intact virions from oyster extracts to a volume and quality compatible with reverse transcriptase polymerase chain reaction (RT-PCR). Virus-seeded oyster extracts processed by adsorption-elution-precipitation were further cleaned and concentrated by Freon extraction, polyethylene glycol (PEG) precipitation, and cetyltrimethyl ammonium bromide (CTAB) precipitation to reduce sample volumes to 100 µl and remove RT-PCR inhibitors. Total virus recoveries were 10% for poliovirus and 20-40% for HAV. Direct RT-PCR detection was possible at levels of 78 pfu polio and 295 pfu HAV. Thus, progress has been made in developing a rapid, sensitive, and effective method to process oysters for RT-PCR detection of enteric viruses at naturally occurring low levels.

AUTOMATED ELISA DETECTION OF *LISTERIA* FROM MEAT AND POULTRY PRODUCTS USING THE VIDAS SYSTEM

J.S. Bailey*, Research Microbiologist and N.A. Cox, USDA, ARS, Russell Research Center, P. O. Box 5677, Athens, GA 30613

The VIDAS® automated immunoanalysis system which combines the ELISA technique with a final reading using fluorescence (ELFA) technology was used to detect the presence of *Listeria* spp. in meat and poultry products. After 48 hr in enrichment broths, samples are boiled for 15 min, loaded in the VIDAS® and automatically run in 45 min. *Listeria monocytogenes* inoculated into 25 gm of hot dogs were correctly identified in all samples with 0.2 or 2.0 cells/gm and in 2 of 5 samples with 0.02 cells/gm initial inoculum. Sixty raw processed chicken carcasses were analyzed for the presence of naturally occurring *Listeria* using the procedures of USDA. There were 27 of 60 samples with confirmed *Listeria* as identified by Fraser broth positive, MOX plate positive, CAMP test and Micro-ID *Listeria*. The VIDAS® identified 20 of these 60 samples as *Listeria* positive. The VIDAS® and other currently available ELISA tests for *Listeria* require between 10^5 and 10^6 *Listeria*/ml of broth, and the failure to detect the 7 positives is directly related to competitors from raw chicken suppressing the growth of *Listeria*. Improvements in enrichment techniques for raw products are needed to assure minimal growth.

USE OF IMMUNOMAGNETIC CAPTURE ON BEADS TO RECOVER *LISTERIA* FROM ENVIRONMENTAL SAMPLES

B.A. Mitchell, J.A. Milbury, A.M. Brookins and Barb Jackson, Ph.D., Director of Pathogen Research, VICAM, 29 Mystic Avenue, Somerville, MA 02145

Most methods for isolation of *Listeria* from food or environmental samples employ selective agents, which can kill injured *Listeria*. Use of selective agents can be minimized if *Listeria* are subjected to immunomagnetic isolation, by using microscopic magnetic beads. Such magnetic beads are coated with antibodies directed against the target organism, and the bound organisms are subsequently isolated in a magnetic field.

Using magnetic beads coated with antibodies directed against *Listeria*, isolation of *Listeria* from environmental samples was achieved within hours. Isolation was coupled to a second stage of *Listeria* growth and immunological characterization, resulting in a total test time of 24 hours. Immunomagnetic isolation and characterization of *Listeria* allowed their detection in 100% of the samples, at contamination levels where a standard cultural method gave detection in 36% of the samples. At lower levels of contamination, immunomagnetic isolation allowed detection of *Listeria* in 58% of the samples, while the cultural method failed to detect *Listeria* in any samples. Because immunomagnetic isolation did not rely on enrichment, the number of *Listeria* colonies isolated was related to the original level of contamination.

ENHANCED RECOVERY AND ISOLATION OF *SALMONELLA* USING A NOVEL CULTURE AND TRANSFER DEVICE

Karl F. Eckner*, Research Scientist, Wendy A. Dustman, Anna A. Rys-Rodriguez, Jay Myrick, and Richard B. Smittle, Silliker Laboratories Research, 1304 Halsted Street, Chicago Heights, IL 60411

A novel transfer-inoculation device for improved detection of *Salmonella* in food and environmental samples was evaluated. Samples were prepared and analyses performed according to BAM/AOAC procedures. The only modification to the standard cultural procedures was utilization of the transfer-inoculation device. A total of 504 food and environmental samples from 20 separate trials consisting of 11 foods or sample types were analyzed for *Salmonella*. Detection of *Salmonella* was improved by >43% compared to the standard BAM/AOAC cultural method without inclusion of the device. A second blind field test in a routine testing laboratory used naturally contaminated and intentionally *Salmonella*-contaminated samples. To date, a total of 226 food and environmental samples have been analyzed for *Salmonella*. Detection of *Salmonella* with the transfer-inoculation device was improved by >25% compared to the standard BAM/AOAC cultural

method without inclusion of the device. Performance improvement was statistically significant ($p \leq 0.0001$) in both set of trials. There were a total of 4 false-negative results with the transfer-inoculation device and 22 false-negative results for the standard cultural methods in the field test.

ENZYME IMMUNOASSAY FOR THE DETECTION OF STAPHYLOCOCCAL THERMONUCLEASE IN FOODS

Paul F. Bina*, Robert H. Deibel, Kristin A. Hedlof, William L. Rose and Raoul F. Reiser, Toxin Technology/Deibel Labs, 7165 Curtiss Avenue, Sarasota, FL 34231

Staphylococcal thermonuclease (TNase) is an extracellular product produced by coagulase positive Staphylococci. TNase presence in foods is used as an indicator of current or previous Staph. contamination. An enzyme immunoassay (EIA) was developed to detect as little as 0.1ng TNase per ml of food extract. The EIA uses an affinity purified rabbit anti-TNase IgG as the capture antibody. The detection antibody is an affinity purified rabbit anti TNase IgG conjugated to horseradish peroxidase. This assay was performed on a variety of food products and food products spiked with either a quantitated amount of TNase or with TNase producing Staph. The results were then compared with the traditional plate-activity method. The results indicated that not only is the EIA method more sensitive, it also has more versatility in the type of foods it can be used to screen. Results from both methods are available in less than 4 hours.

OCCURRENCE OF FALSE POSITIVE TESTS FOR STAPHYLOCOCCAL ENTEROTOXIN USING THE TECRA KIT

R.H. Deibel*, P.F. Bina, W.L. Rose, K.A. Hedlof, R.F. Reiser, Deibel Laboratories/Toxin Technology, 7165 Curtiss Avenue, Sarasota, FL 34231

Ninety-five of 147 raw, in-process and finished (heated shelf-stable or cold packed) pickles tested positive for staphylococcal enterotoxin using the TECRA kit. Some brines of various ages and pickle relish were also positive for enterotoxin using TECRA. All samples were negative for toxin using the SET-EIA kit. All samples were negative for thermostable nuclease. Thirty-five of the TECRA positive samples were negative for toxin using the FDA microslide procedure. Some other plant foods gave a positive TECRA test. Subsequently, it was demonstrated that the false-positive TECRA tests were due to the natural peroxidase in the plant foods thus mimicking the peroxidase conjugate in the test. In contrast, the SET-EIA conjugate is a phosphatase. This study indicated that extreme care must be exercised when interpreting results obtained with the TECRA kit.

TIME/TEMPERATURE RESPONSE OF ACID PHOSPHATASE IN COOKED BROILER BREAST USING A FLUOROMETRIC ASSAY

C.E. Davis*, Research Food Technologist, W.E. Townsend, and C.E. Lyon, USDA, ARS, Russell Research Center, P. O. Box 5677, Athens, GA 30613

U.S. Department of Agriculture, Food Safety and Inspection Service requires uncured poultry to be heat processed to 160° F/71.1° C if labeled fully cooked. Breast acid phosphatase (ACP) activity at five end-point temperatures (EPT) and three dwell times was measured by a fluorometric assay. The experiment was replicated two times with triplicate ACP instrument readings. A time/temperature curvilinear decrease in mean (N=12) ACP activity occurred. There were time/temperature differences ($P<.05$) among EPT's and dwell times. EPT means ± S.E. for ACP activity (mU/Kg) at 62.8, 65.6, 68.3, 71.1, 73.9°C, and 0, 15, 30 min dwell were as follows: 11915.3 ± 98.2, 5387.4 ± 193.3, 1669.5 ± 24.0, 706.6 ± 27.0, 573.2 ± 23.0; 3582.8 ± 66.6, 1191.9 ± 43.9, 443.2 ± 22.6, 388.6 ± 13.9, 318.7 ± 22.4 ; 1881.5 ± 42.6, 584.28 ± 17.1, 346.2 ± 11.9, 298.6 ± 9.64 ± 289.9 ± 21.6, respectively. This procedure provides a rapid (3 min instrument time), sensitive analytical method for quality assurance process control technicians or regulatory analysts to monitor EPT in cooked poultry.

CHARM PESTICIDE TEST: RAPID SCREENING METHOD FOR THE DETECTION OF ORGANOPHOSPHATE AND CARBAMATE PESTICIDES FOR WATER, DAIRY PRODUCTS, FRUITS, VEGETABLES AND OTHER FOOD PRODUCTS

Steven Saul*, E. Zomer and S. E. Charm, Charm Sciences, Inc., 36 Franklin Street, Malden, MA 02148-4120

A rapid screening method has been developed for the single test detection of cumulative organophosphate and carbamate pesticides in water and food materials. Results are measured using the Charm II system. In water, no extraction is required and assay time is 15 min. For raw/pasteurized milk a pretreatment step increases assay time to 20 min. Other food materials require an additional simple two phase extraction and drying procedure of about 15 min. Limit of detection in water for more than 20 representative pesticides range from 0.1 to 20 ppb. For some organophosphates the natural active metabolites that are found in the field are detected with greater sensitivity than the parent compound. For example, guthionoxon, malaoxon, methyl paraoxon, and paraoxon are detected in water at 0.1 ppb, 0.7 ppb, 0.1 ppb and 0.3 ppb, respectively. These are the active metabolites to guthion, malathion, methyl parathion and parathion, respectively. Sensitivity of the test may be adjusted by dilution of the matrix.

For water, a survey of bottled water and city water samples were found negative with ethion at 2.5 ppb used as the control detection level. Market milk samples and raw milk samples over a two week period were tested and using a control point of 30 ppb ethion all samples were detected as negative. Various plain yogurts and fruit yogurts from various supermarkets were tested. All plain yogurts tested negative. For fruit yogurts 4 samples out of 20 were positive with the control point set at 100 ppb ethion. Apples were tested from local orchards and supermarkets. Using 100 ppb ethion as the control point there were 4 positives out of 23 apples tested.

Confirmation of positive samples is being performed using HPLC. One yogurt sample demonstrated 4 positive peaks by HPLC analysis. Further HPLC confirmation and confirmation by HPLC and mass spectrophotometry is ongoing.

FUMONISIN SYMPOSIUM

FUMONISIN PRODUCTION BY TOXIGENIC STRAINS OF *FUSARIUM MONILIFORME* AND *FUSARIUM PROLIFERATUM* IN CORN

Charles W. Bacon* and P. E. Nelson, Toxicology and Mycotoxin Research Unit, Russell Research Center, USDA/ARS, Athens, GA

The fungi *F. moniliforme* Sheldon and *F. proliferatum* (Matsushima) Nirenberg produce a series of toxins on corn which include the fumonisins of which fumonisin B_1 and B_2 are considered to have cancer promoting activity. Both fungi produce similar ratios of fumonisin B_1 to B_2. Other mycotoxins produced include moniliformin, fusarin C, and fusaric acid. The distribution of these two fungi is generally similar, although *F. proliferatum* is isolated more frequently from sorghum than corn. They occur worldwide on other food crops such as rice, sorghum, millet, and several fruits. Both fungi are ear rot pathogens of corn, thus mycotoxins may be produced under field conditions, although they may also occur in storage. One or both fungi may have a frequency of occurrence of 90% or higher in corn; 90% of the *F. moniliforme* isolates are toxic. Fumonisin B_1 has been shown to be responsible for most of the toxicological effects observed from ingesting toxigenic isolates of these fungi.

THE TOXICITY AND ROLE OF FUMONISINS IN ANIMAL DISEASES AND HUMAN ESOPHAGEAL CANCER

William P. Norred, Supervisory Pharmacologist and Research Leader, Toxicology and Mycotoxin Research Unit, Russell Research Center, ARS/U. S. Department of Agriculture, PO Box 5677, Athens, GA 30613

Fumonisins are secondary metabolites of *Fusarium moniliforme*, *F. proliferatum*, and several other *Fusaria* that commonly contaminate corn. Only recently discovered in 1988, these mycotoxins appear to be the causative agents of several toxicoses in animals that result from ingestion of moldy corn or corn based feeds. The syndromes vary considerably among the different species affected and include brain lesions in equids, lung edema in swine, and nephrotoxicity, hepatotoxicity and hepatocellular carcinoma in laboratory rats. There is accumulating evidence that suggests that *F. moniliforme* and fumonisins may also be responsible for esophageal cancer in humans in certain areas of the world where moldy corn is frequently consumed. Studies are currently underway to determine the extent of the hazards posed by fumonisins, and whether controls in the form of regulatory action levels may be necessary.

MECHANISMS OF FUMONISIN TOXICITY AND CARCINOGENESIS

Ronald T. Riley*, Research Pharmacologist and Alfred H. Merrill, Jr., Toxicology and Mycotoxin Research Unit, U.S. Department of Agriculture, ARS, Russell Research Center, PO Box 5677, Athens, GA 30613

What are the molecular events that fumonisin (FB)-induced porcine pulmonary edema syndrome and equine leucoencephalomalacia have in

common? Do these animal diseases relate mechanistically to FB-induced toxicity in rats? While the answers are far from complete, there is considerable data which indicate that disruption of sphingolipid biosynthesis may play an important early role in all of these conditions. Soon after animals are exposed to FB's, there is a dramatic increase in the free sphingoid base, sphinganine, in tissues, serum, and/or urine. Free sphingosine levels also become elevated. This elevation in free sphingoid bases is due to FB inhibition of the enzyme sphinganine (sphingosine) N-acyl transferase (ceramide synthase). It is hypothesized that disruption of sphingolipid metabolism at this level is an early molecular event in the onset and progression of all the diseases associated with consumption of FB contaminated feeds.

METHODS FOR DETECTION AND QUANTITATION OF FUMONISINS IN CORN AND CEREAL PRODUCTS

Larry G. Rice*, Chemist, and P. Frank Ross, U.S. Department of Agriculture, APHIS, NVSL, 1800 Dayton Road, Box 844, Ames, IA 50010

Fumonisins occur in a wide variety of animal feed and human foods. Fumonisins are the causative agent of equine leukoencephalomalacia (ELEM) and porcine pulmonary edema syndrome (PPE), and have been associated with human esophageal cancer. The analytical method of choice for most samples suspected in field toxicosis has been high performance liquid chromatography (HPLC) using fluorescence detection. The present work describes the evaluation of the o-phthalaldehyde derivatives of fumonisin B_1 (FB_1), fumonisin B_2 (FB_2), and fumonisin FB_3, (FB_3) and their baseline resolution using an isocratic mobile phase. The hydrolyzed forms of fumonisin FB_1; partially hydrolyzed FB_1 ($PHFB_1$) and fully hydrolyzed FB_1 (HFB_1), is also accomplished. Results of analyses of corn and corn based foods are presented.

INCIDENCE AND LEVELS OF *FUSARIUM MONILIFORME, FUSARIUM PROLIFERATUM* AND FUMONISINS IN CORN BASED FOODS

Lloyd B. Bullerman*, Professor, and W.-Y. J. Tsai, Department of Food Science and Technology, University of Nebraska, Lincoln, NE 68583-0919

Fusarium moniliforme and *Fusarium proliferatum* are common to all corn growing regions and are frequently found as contaminants of corn where there are no outward signs of mold infection of the kernel. Also, fumonisins have been found in low background levels in good quality corn. This raises concerns about the incidences of *F. moniliforme* and *F. proliferatum* and levels of fumonisins that may be present in food grade corn and popcorn, and corn based foods. The highest levels of fumonisins have been found in damaged corn (>500 µg/g) and corn screenings (125 µg/g). Lower levels have been found in undamaged (good quality) corn (<1 to 4 µg/g). Surveys of corn based human foods have found fumonisins in corn meal (0.5 to 2.0 µg/g), corn grits (0.14 to 0.27 µg/g), corn bran cereal (0.13 to 0.33 µg/g), hominy (0.06 µg/g) and popcorn (0.01 to 0.06 µg/g). Corn flakes and corn pop breakfast cereals and corn chips were negative, while tortilla chips and tortillas had low levels in some samples (0.03 to 0.12 µg/g). This paper will review the available data on the incidence of *F. moniliforme* and *F. proliferatum*, and levels of fumonisins in corn and corn based foods.

CAMPYLOBACTER UPDATE SYMPOSIUM

Sponsored by the International Life Sciences Institute

HUMAN CAMPYLOBACTERIOSIS: CLINICAL AND EPIDEMIOLOGICAL ASPECTS

Patrick De Mol, M.D., WHO Collaborating Centre for Enteric Campylobacter, Hopital Universitaire Saint- Pierre, 322 Rue Haute, 1000 Brussels Belgium

Campylobacter enteritis is the most frequent form of acute bacterial diarrhea in developed countries. It affects people of all ages and is prominent in young adults. The infection is seasonal in temperate climates. About twice as many infections occur in summer than in winter. In developing countries the disease is confined to young children who develop immunity early in life through repeated exposure to infection. Acute enterocolitis is the most common presentation of Campylobacter infection but symptoms and signs are not so distinctive that the physician can differentiate it from illness caused by other organisms. The frequent finding of dysenteric stools suggests that mucosal damage due to an invasive process analogous to that seen in Shigellosis is important in the pathogenesis. Indeed the fact that many patients have erythrocytes and leucocytes in their stools suggest colonic involvement. Campylobacter enteritis has a very good prognosis and isolation of the organism from the stools does not warrant chemotherapy. In the absence of chemotherapy, faeces remain positive for about 2 to 7 weeks after the illness. Erythromycin or one of the newer macrolides when started within 4 days of onset of symptoms has a clinical benefit and shortens the faecal excretion of Campylobacter. If abdominal pain is severe or the possibility of complication exists, it is preferable to administer an antibiotic. Erythromycin stearate 500 mg twice a day for adults, and erythromycin ethylsuccinate, 40 mg/kg/day for children, for 5 days are recommended. Campylobacter enteritis is a zoonosis of worldwide distribution and there may be many pathways by which humans can become infected. Poultry constitutes the largest potential source of food-borne infection in humans. Infection can be acquired by handling the raw product, in the kitchen or by consuming it raw or undercooked. Raw or undercooked beef hamburgers, sausages and clams, as well as poultry have been implicated in outbreaks of Campylobacter enteritis. Massive outbreaks affecting several thousand people have been caused by the distribution of raw or inadequately pasteurized milk or inadequately treated water. The high prevalence of the organism in the tropics and its short incubation period are reflected in its frequence as a cause of traveller's diarrhea. Prevention of Campylobacter infection depends upon the purification of all water supplies, the heat treatment of all milk sold for human consumption, the hygienic handling of all raw meats (especially poultry in kitchens) and the control of infection at all stages of poultry production.

CAMPYLOBACTER: A EUROPEAN PERSPECTIVE

Michael F. Stringer, Head of Microbiology Department, Campden Food and Drink Research Association, Chipping Campden, Gloucestershire, GL55 6LD, United Kingdom

In England and Wales the number of reported cases of Campylobacteriosis increased steadily from 12,168 in 1981 to 34,552 in 1990 and by 1992 had risen to 38,570 (provisional figure). Since 1981, Campylobacter has been more prevalent than Salmonella in England and Wales and is the most frequently isolated pathogen from cases of acute infectious diarrhea.

A similar picture exists in Scotland where the number of cases rose from 1,887 in 1981 to 4,917 in 1992. Where they are available, figures for other European countries will be presented.

Information will be presented on the sources and transmission of infection. The microbiological factors influencing growth and survival in foods will also be discussed, with reference to the application of predictive microbiology (mathematical modelling).

Issues of concern and interest to the food industry will be discussed in relation to possible methods for control and prevention of Campylobacter infection in humans.

CAMPYLOBACTERS AND THEIR EPIDEMIOLOGICAL MARKERS

Hermy C. Lior, Chief, Laboratory Center for Enteric Pathogens, Laboratory Centre for Disease Control, Tunney's Pasture, Ottawa, Ontario, K1A 0L2 Canada

Campylobacters are a leading cause of human diarrheal disease around the world and are also widely distributed among animals, birds and the environment. Food animals and especially poultry and turkeys are major reservoirs of these organisms and also the vehicle for human infections in addition to unpasteurized milk and contaminated water.

First described in Germany in 1886 by Th. Escherich, it was not until early '70s that we were able to isolate these organisms from human diarrheal disease. Certain species pathogenic for animals such as *Vibrio fetus, (Campylobacter fetus* spp *fetus* and *Campylobacter fetus* spp *venerealis)* were known to veterinary people since the beginning of the century.

Today we recognize about 11 species and 4 subspecies of Campylobacters associated mostly with human disease, among them *C. jejuni* ssp *jejuni, C. coli, C. lari* and *C. upsaliensis.*

C. jejuni ssp *jejuni* accounts for most human infections and are also isolated from poultry, cattle, sheep, dogs and birds. *C. upsaliensis* is increasingly isolated from human disease and is commonly found in pets - dogs and cats. Other species such as *C. coli* account from about 10-25% of human isolations and *C. lari* for less than 1%. *Campylobacter butzleri* now known as *Arcobacter butzleri* is a newly described species which appears quite common in the environment, poultry and porcine and is also isolated from human diarrheal disease.

Overall Campylobacters represent the most commonly isolated bacteria from diarrheal disease in many countries, surpassing the isolation of Salmonellae.

The differentiation among these organisms require a variety of markers necessary for epidemiological investigations. Traditional methods such as serotyping, biotyping and phagetyping schemes have been developed and

newer techniques such as DNA fingerprinting using arbitrary primers, Pulse-Field Gel Electrophoresis and ribotyping provide a variety of phenotypic and genotypic characteristics used in epidemiological investigations.

CAMPYLOBACTER JEJUNI: THE U. S. DEPARTMENT OF AGRICULTURE PERSPECTIVE

Ann Marie McNamara, Director, Microbiology Division, Food Safety and Inspection Service, U.S. Department of Agriculture, 300 12th Street, S.W., Room 410 Annex, Washington, DC 20250

In three separate sessions, the USDA perspective on three foodborne pathogens will be presented. Included in the presentations will be current data from the Food Safety and Inspection Services' testing program for *Listeria monocytogenes* in cooked, ready-to-eat meat and poultry products, data on the recovery of *L. monocytogenes, E. coli* O157:H7, and *Campylobacter jejuni* from raw product surveys, current USDA methods for identification of these bacteria including results of USDA methods development work, and a brief overview of USDA's participation in the recent *E. coli* O157:H7 outbreak in Washington State.

CAMPYLOBACTER JEJUNI: THE U. S. FOOD AND DRUG ADMINISTRATION PERSPECTIVE

Joseph M. Madden, Director, Division of Microbiology, Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, 200 C Street, S.E., Washington, DC 20204

Three human foodborne pathogenic bacteria have dominated the food safety literature for the past 10-15 years: *Listeria monocytogenes, Escherichia coli* O157:H7, and *Campylobacter jejuni*, and all have been found in food products regulated by the FDA. *Campylobacter jejuni* is now considered the most frequent cause of bacterial diarrhea in the U.S., the major vehicles of transmission to man being drinking water, raw milk, and improperly cooked/handled poultry. A seafood outbreak involving clams has recently been described. The ingestion of as few as 200 organisms may cause gastroenteritis in susceptible individuals, possibly accounting for the large number of cases in the United States.

INTERNATIONAL PERSPECTIVES ON *ESCHERICHIA COLI* O157:H7

Sponsored by the International Life Sciences Institute

E. COLI O157:H7 TIME CAPSULE: WHAT DO WE KNOW AND WHEN DID WE KNOW IT

Marguerite A. Neill, Infectious Disease Division, Brown University School of Medicine and Memorial Hospital of Rhode Island, 111 Brewster Street, Pawtucket, RI 02860

Few newly emerging pathogens have had as dramatic an entrance as *E. coli* O157:H7. In the decade since its initial description as a causative agent of human disease, there has been a rapid outpouring of information on the biochemical and genetic characterization of *E. coli* O157:H7, a preliminary assessment of the epidemiology of human and animal infection and an appreciation of the spectrum of clinical illness associated with infection. This initial wave of information has set the stage for tackling the more difficult questions on the modes and efficiency of transmission, the pathogenesis of diarrheal disease and the complications of hemolytic uremic syndrome, and the ecological niche of *E. coli* O157:H7 in nature and the food chain. The need to understand these areas is highlighted by their direct relationship to designing effective control and prevention strategies for this important new pathogen.

E. COLI O157:H7 AND VEROTOXIGENIC *E. COLI*

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Enterohemorrhagic *Escherichia coli* or Verotoxigenic *E. coli* represent a recently described group of pathogens which produce a cytotoxin to several tissue culture lines such as Vero and HeLa cells. This toxin described by Konowalchuk et al. in Canada in 1977 as Verocytotoxin (VT) also known as Shiga-like toxin (SLT), has been described in several serotypes of *E. coli* isolated from human and nonhuman sources.

To date we recognize 4 main types associated with human disease: VT1 (SLT-1), VT2 (SLT-2), VT2Vh (SLT2vc) and VTevh (SLT-2va) and 2 subtypes of VT2vh: VT2va and VT2vb. Another verotoxin, VTe (SLT2vp) has been associated with piglet disease. Verotoxigenic *E. coli* have been isolated from human cases of watery and/or bloody diarrhea and also from cases of Hemolytic Uremic Syndrome - a kidney failure complication of infections with Verotoxigenic *E. coli*.

E. coli serotype O157:H7 isolated from outbreaks of hemorrhagic colitis in 1982 in USA and Canada have been shown by us to produce verotoxins. To date more than 100 different serotypes belonging to over 50 *E. coli* serogroups have also been shown to produce a variety of verotoxins.

Contaminated ground meat, unpasteurized milk and contaminated water have been the main vehicles of human infection. Contaminated cider has been implicated in one outbreak in Canada and recently in one outbreak in USA.

Consumption of improperly cooked hamburgers, drinking raw milk and person-to-person spread in institutions such as Day-Care Centers are responsible for the many cases reported in several countries such as UK, Germany, Argentina, USA and Canada where over 1600 laboratory isolations have been reported in 1992.

Complete serotyping, toxin typing and phagetyping are some of the techniques which can provide useful markers in epidemiological investigations.

E. COLI O157:H7 OUTBREAK IN THE WESTERN UNITED STATES

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In January 1993, a large outbreak of *Escherichia coli* O157:H7 infection was identified in Washington State. Case control investigation initiated by County and State Epidemiology offices demonstrated that consumption of hamburgers at multiple outlets of a single restaurant chain was a risk factor for infection. Smaller antecedent or synchronous outbreaks in California, Nevada, and Idaho were subsequently identified, and the same vehicle was incriminated. Microbiologic investigation yielded *E. coli* O157:H7 from hamburger patties in the stores, and insepction demonstrated inadequate cooking practices. 614 patients met the case definition, including 491 culture positive patients, and 123 patients with hemorrhagic colitis and/or hemolytic uremic syndrome (HUS) whose cultures were negative (n=75) or not performed (n=48). 3.1 clinically apparent infections resulted from every 1000 regular hamburgers purchased, and approximately 250,000 contaminated patties were recalled. 58 (11.8%) cases have been determined to be secondary to contact with a primarily infected case, defined to be a person who consumed a hamburger at a Washington outlet of the chain in question. The median age of the cases was 7.5 years (range 0-74 years). At least 35 patients developed HUS, and 3 died. This outbreak demonstrates the virulent nature of *E. coli* O157:H7 infection, confirms the impression that few bacteria can cause human disease, and demonstrates the value of an active foodborne disease surveillance system and a rapid epidemiologic response to an outbreak.

*E. COLI O*157:H7 THE U. S. DEPARTMENT OF AGRICULTURE PERSPECTIVE

Ann Marie McNamara, Director, Microbiology Division, Food Safety and Inspection Service, U.S. Department of Agriculture, 300 12th Street, S.W., Room 410 Annex, Washington, DC 20250

In three separate sessions, the USDA perspective on three foodborne pathogens will be presented. Included in the presentations will be current data from the Food Safety and Inspection Services' testing program for *Listeria monocytogenes* in cooked, ready-to-eat meat and poultry products, data on the recovery of these three pathogens from raw product surveys, current USDA methods for identification of these bacteria including results of USDA methods development work, and a brief overview of USDA's participation in the recent *E. coli* O157:H7 outbreak in Washington State.

E. COLI O157:H7: THE U.S. FOOD AND DRUG ADMINISTRATION PERSPECTIVE

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Three human foodborne pathogenic bacteria have dominated the food safety literature for the past 10-15 years: *Listeria monocytogenes, Escherichia coli* O157:H7, and *Campylobacter jejuni*, and all have been found in food products regulated by the FDA.

E. coli O157:H7, infections of humans were first described in 1982 following a large outbreak of hemorrhagic colitis associated with the con-

sumption of hamburger from a fast food chain. It has recently again gained notoriety following another outbreak of gastroenteritis in the western U.S. also associated with the consumption of hamburgers purchased at a fast food chain. In addition to outbreaks of human illness attributed to ground meats, outbreaks have been associated with the consumption of apple cider (Massachusetts, 1991), raw milk (Oregon, 1992-1993), and an epidemiological linkage has been established between the consumption of food commodities containing mayonnaise and eleven cases of hemorrhagic colitis (Oregon, 1993). The intriguing characteristic of this microbe is its ability to survive relatively low pH (3.8-4.2) for a significant period of time (3-4 weeks). Data indicate that a relatively low infectious dose is required for this microbe to establish an infection in young children.

TECHNICAL SESSION — GENERAL FOOD MICROBIOLOGY

COMPARISON OF AFLATOXIN PRODUCTION IN MODIFIED CZAPEK'S SOLUTION AGAR, AFPA, AND DYE MEDIA

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Aflatoxins are potent mycotoxins and carcinogens produced by strains of *Aspergillus flavus*, *A. nomius*, and *A. parasiticus*. These mycotoxins continue to be a public health hazard due to their high toxicity and the ubiquitous nature of aspergilli in the environment as well as in the food chain. Modified Czapek's Solution Agar and *Aspergillus flavus/A. parasiticus* agar (AFPA) have been used for screening of potential aflatoxin producing aspergilli. However, these agars are time consuming to prepare. Recently we developed simple basic violet agars such that only species of *Aspergillus* and/or *Penicillium* will grow. We compared all of these agars for the ability to grow aflatoxin-producing strains of aspergilli. Veratox™ test kits (NEOGEN) and Ultraviolet light were used to screen for aflatoxin.

Representative strains known from mycotoxin investigations at the Northern Regional Research Laboratory to be either aflatoxin-positive (NRRL 465, NRRL 2999, NRRL 3251, NRRL 5520) or aflatoxin-negative (NRRL 1957) as well as various aspergilli isolated from contaminated food & feed were examined. All three types of agar were suitable for growing toxin-producing strains of aspergilli, although preparation of the AFPA or dye media was easier and less time consuming than preparation of the Modified Czapek's Solution Agar.

INFLUENCE OF AFLATOXIN AND NUTRIENT CONCENTRATION ON THE DEGRADATIVE ABILITY OF *FLAVOBACTERIUM AURANTIACUM*

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Flavobacterium aurantiacum has been demonstrated to degrade ^{14}C-labeled aflatoxin B_1 (^{14}C-B_1) in phosphate buffer. This study was conducted to determine the effect of aflatoxin B_1 (AFB_1) concentration and presence of nutrients (tryptic soy broth) on the ability of *F. aurantiacum* to degrade AFB_1. Radiolabeled AFB_1 was used to trace metabolism. Following incubation with ^{14}C-B_1, the total cell pellet, chloroform-and water-soluble fractions of supernatant fluid, and CO_2 were analyzed for radioactivity. Ultraviolet absorption maxima of non-radiolabeled samples were measured using a scanning spectrophotometer to determine the spectra of AFB_1 degradation products. Presence of non-radiolabeled AFB_1 (3 µg/ml) reduced the degradation of ^{14}C-B_1. Evolved $^{14}CO_2$ decreased by almost 50% when non-radiolabeled AFB_1 was also present. In addition, added nutrients increased AFB_1 degradation. The appearance of an ultraviolet absorption maximum at 404 nm with the concurrent disappearance of the AFB_1 absorption maximum at 363 nm was noted in water-soluble fractions after exposure to *Flavobacterium*. Control samples containing no cells showed no change in the AFB_1 absorption spectrum. These data confirm earlier studies reporting the ability of *F. aurantiacum* to degrade AFB_1 to water-soluble products and suggests additional energy may enhance degradation.

DETERMINATION OF CYTOSOLIC AFLATOXIN B_1-DEGRADING ACTIVITY OF *FLAVOBACTERIUM AURANTIACUM*

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Aflatoxin contamination of agricultural commodities poses a safety risk to humans and livestock. Live cells of *Flavobacterium aurantiacum* (*Exiguobacterium aurantiacum*) have been shown to irreversibly remove aflatoxins from broth and certain foods. Removal of aflatoxin B_1 by cellular fractions was investigated. A late log phase culture of *F. aurantiacum* was centrifuged and the cell-free extract tested for aflatoxin-degrading ability. After 60 h incubation at 30° C, HPLC analysis indicated only 16% of the initial aflatoxin B_1 had been removed (2.0 µg/ml initial concentration). The cell pellet was separated into a cytosolic and a membrane fraction by sonication and ultracentrifugation. At an initial aflatoxin level of 5.0 µg/ml, these fractions removed 99 and 27% of the toxin, respectively, during 48 h at 30° C. The cytosol fraction of *F. aurantiacum* may be a valuable source of a constitutive enzyme which degrades aflatoxin.

LEVEL OF *CAMPYLOBACTER* SPP. ON BROILER FARMS AND AFTER CHICKEN TRANSPORT

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Levels of *Campylobacter* spp. colonization in ceca and on carcasses of chickens at broiler farms and after transport to a processing facility were determined. Twenty chickens obtained from each of 10 broiler farms were collected from houses containing 6 to 7 week-old birds. Ten chickens were killed at the farm while the other ten were transported in coops to a holding facility and killed after 16-18 h of holding time. Levels of *Campylobacter* spp. were assessed by washing the carcasses in phosphate buffered saline (PBS, pH 7.2) and cecal contents were also enumerated. On the farm, the mean cecal count was $\log_{10}$ 5.44 CFU *Campylobacter* spp./g and after transport the mean was $\log_{10}$ 6.15 CFU. The mean level of *Campylobacter* spp. on chicken carcasses before transport was $\log_{10}$ 4.58 CFU/carcass and after transport was $\log_{10}$ 7.05 CFU/carcass. These increases in levels of *Campylobacter* spp. suggests that transport is a likely contributing factor to the high numbers and prevalence of *Campylobacter* spp. in and on chickens.

INFLUENCE OF SEASON AND STORAGE ON *CAMPYLOBACTER* SPP. CONTAMINATING BROILER CARCASSES

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The frequency and levels of *Campylobacter* spp. associated with broiler chicken carcasses were monitored quarterly, over one year. At three-month intervals, we obtained 50 carcasses from a local processing plant, which had been in continuous operation for at least 12 hours. At each sample interval we monitored 10 carcasses initially and again after 1,3,7, and 10 days of 4°C storage in zippered plastic bags. Both enrichment culture and enumeration on selective media were employed. We observed our lowest initial rate of detection in spring and the highest rate in summer and fall. The levels detected ranged from non-detectable to 600,000 CFU per carcass. Detection of *Campylobacter* spp. was lowest after 10 days of 4°C storage. Cooler months of the year in northeast Georgia corresponded with a reduction in presence and levels of *Campylobacter* spp. associated with broiler carcasses. These reductions could be related to a seasonally diminished presence in source of the organism for the chickens. Detection of the organism was reduced with time under refrigerated storage.

INCIDENCE OF *CLOSTRIDIUM BOTULINUM* IN MODIFIED ATMOSPHERE PACKAGED VEGETABLES

E. Jeffery Rhodehamel*, Research Microbiologist, Timothy Lilly, Jr., Haim M. Solomon, and Donald Kautter, Division of HACCP Programs Food and Drug Administration, 200 C Street, S.W., Washington, DC 20204

The modified atmosphere packaging (MAP) of vegetables may provide an anaerobic environment conducive to *Clostridium botulinum* growth and toxin production. Because of this concern about MAP vegetables, the incidence of *C. botulinum* spores in commercially available, pre-cut MAP vegetables was determined. One-pound packages (454 grams) of MAP vegetables were aseptically opened in a laminar flow hood. Each package was equally divided (approximately 150 grams each) among three 1-liter bottles containing 500 ml of freshly steamed and cooled sterile TPGY broth. TPGY broth cultures were incubated at 35° C for 7 days. Positive and negative controls were included with each sample. The broth cultures were tested for toxicity at the end of the incubation period, by using the standard mouse bioassay. The 725 samples analyzed included 337 shredded cabbage, 201 chopped green pepper, 90 mixed vegetable, 35 Oriental salad, 24 shredded cole slaw, 24 Italian salad, 7 carrot, 4 onion, and 3 broccoli. One sample of shredded cabbage, one chopped green pepper, and one Italian salad were positive for the presence of *C. botulinum* Type A spores (0.41% overall incidence rate). Results indicate a low incidence of *C. botulinum* spores in commercially available pre-cut MAP vegetables.

PREVALENCE OF *SALMONELLA* IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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The incidence of *Salmonella* was determined in 30 rainbow trout samples from 25 retail stores in Knoxville, Tennessee. Fifty grams of trout (whole muscle) was selectively enriched for *Salmonella* at 35°C for 24 h in both tetrathionate and selenite-cystine broth and streaked for isolation on brilliant green agar and bismuth sulfite agar. Five samples (16.7%) were found *Salmonella* positive. One sample was found *Salmonella* positive from both tetrathionate and selenite-cystine enrichment, while the other four positive samples were only found from selenite-cystine broth. Aerobic plate counts and coliform counts were also evaluated for each sample. The aerobic plate count ranged from 2.7 to 8.7 log CFU/g with 37% of the samples ≥ 6.0 log CFU/g. The coliform counts ranged from <1 to 5.8 log CFU/g with 37% of the samples ≥ 3.0 log CFU/g.

RATES OF ADHERENCE TO STAINLESS STEEL BY FOODBORNE MICROORGANISMS

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Attachment of microorganisms to food processing surfaces may cause contamination that contributes to food safety concerns and reduced product quality. To determine adherence rates, stainless steel chips (6 mm x 6 mm) were immersed in tryptic soy broth (TSB) or diluted TSB (dTSB) containing either *Salmonella typhimurium*, *Escherichia coli* O157:H7, *Listeria monocytogenes* or *Pseudomonas fragi* in lag, log or stationary phase. Chips were removed at selected times up to 30 min. To enumerate the attached cells, the chips were rinsed, stained with acridine orange and viewed using epifluorescent microscopy. The highest initial adherence was seen for *P. fragi* (dTSB, stationary) and *S. typhimurium* (dTSB, stationary), however, little increase in attached cells was seen over 30 min. The lowest rates of adherence were seen for *E. coli* (all conditions) and *S. typhimurium* (TSB, stationary). The microorganisms with the highest rates of attachment may be of the most concern in a food processing environment.

BACTERIA ON BEEF BRISKETS AND GROUND BEEF: ASSOCIATION WITH SLAUGHTER VOLUME AND ANTEMORTEM CONDEMNATION

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Aerobic plate counts of 3455 brisket and 1370 ground beef samples were examined for association with slaughter volume in 547 U.S. beef slaughter establishments. High volume beef slaughter establishments controlled total aerobic bacteria counts on briskets and ground beef more effectively than low volume establishments. Lower APCs may have resulted from measures taken to prevent contamination, effective decontamination, obtaining cattle from fewer sources, specialization in slaughter procedures, and less variation in procedures used. *Salmonella* contamination increased as antemortem condemnation increased in establishments that slaughter calves. Slaughter volume was not correlated with contamination on briskets or ground beef with *Salmonella*. *Salmonella* contamination was more closely associated with the health of animals brought to slaughter than with conditions in the beef slaughter establishments.

COMPRESSED AIR, CITY WATER AND DUST AS SOURCES OF CONTAMINATION OF A DAIRY ASEPTIC PROCESSING SYSTEM

Corey Lerbs, 6125 Camden Avenue N., Brooklyn Center, MN 55430

Five species of strictly aerobic bacteria were repeatedly isolated from aseptically processed milk or soy-milk spoilage tests at Tetra Pak's pilot plant. *Pseudomonas syringae* was found throughout the compressed air system, *Pseudomonas aeruginosa* was prominent in city water. *Bacillus cereus*, *B. polymyxa*, and *B. circulans* were prominent in dust. None of the five isolates could be isolated from the unprocessed milks. All five grew on rubber (EPDM) gaskets immersed in phosphate buffer and produced mucoid colonies on sucrose media. Cleaning buckets contained only facultative bacteria including *Enterobacter aerogenes*. Equipment samples showed no sign of the bacteria causing spoilage of aseptically processed milk or soy milk.

BAKING EQUIPMENT STANDARDS AND GENERAL SANITATION IN BAKING OPERATIONS SYMPOSIUM

WHAT IS BISSC?

Sigismondo DeTora* and Frank Goley, Nabisco Biscuit Company, 200 DeForest, P. O. Box 1944, East Hanover, NJ 07936-1944

A short history of how and why BISSC was formed. We will examine its initial and on going function in the baking industry. How were the standards formulated and how they are enforced. We wish to convey the importance that BISSC places on sanitation standards and the "BASIC CRITERIA." Finally we will look at where BISSC is going in the future.

SANITARY DESIGN — A MIND SET

Donald J. Graham, Sverdrup Corp., 801 N. 11th Street, St. Louis, MO 63101

A review of sanitary design concepts starting with regulations, site selections, structural and landscaping. Sanitary design features inside the food processing facility will be shown with both good and bad features including equipment, personnel facilities and process areas. The presentation will stress awareness and necessity for sanitary design in food processing facilities.

OSHA REGULATORY REQUIREMENTS

James Dykes, American Institute of Baking, 1213 Bakers Way, Manhattan, KS 66502

- Overview of current standards, new standards and rule making that will affect your industry.
- Trends in enforcement: What standards are most enforced? How much money is OSHA planning on collecting from business?
- Staffing levels and OSHA departmental organization that affects all businesses.
- What should you do when OSHA comes?
- What should you do before OSHA comes?
- A projection from OSHA's current position to where they intend to go in 1994 and beyond.

HAZARD ANALYSIS AND CRITICAL CONTROL POINTS (HACCP): CONCEPT AND USE

Ron Vail, President, Food Safety Systems, Inc., 3778 Falcon Way, Eagan, MN 55123

Hazard Analysis Critical Control Point (HACCP) pronounced "Hassip" is a method of systematic analysis to determine the location of conditions and situations in food processing and handling facilities where actual or potential food safety issues exist or could exist if adequate controls are not implemented.

One challenge is to understand the components of HACCP and effectively develop, implement and integrate HACCP into existing operations.

This presentation will describe the HACCP concept and outline effective development and implementation procedures without significant additional resources. HACCP does not have to be complicated to be functional and more than likely most food operations are already performing most of the procedures. The components which usually need development or strengthening are the supporting documentation, information evaluation processes, determination of appropriate corrective measures and initiating continuous improvement activities.

MICROBIAL CONCERNS OF THE INTERNATIONAL SYMPOSIUM

Sponsored by the International Life Sciences Institute

MICROBIOLOGICAL SAFETY OF FOODS IN EUROPE OF THE NINETIES: WHAT DOES THAT IMPLY?

Michael van Schothorst, NESTEC, Ltd., Nestlé Research Centre, P.O. Box 44, Ch - 1000 Lausanne, Switzerland

Europe is expanding: new countries with different cultures, eating habits, food laws and legal systems are merging. Retailers are becoming more influential in the production and trade of foods and competition between food industries is intensifying. At the same time, consumer expectations and characteristics are evolving. All of these changes have important implications for foods in Europe of the nineties.

With the move towards European unification and the introduction of new legislation, many new questions are being raised. For example, according to EEC directives, the producer is responsible for food safety, and liable for any failures that occur. Application of the HACCP concept is accepted as the best means to assure safety, but who defines "hazards", the producer, the legislator, the retailer or the consumer? What will be the role of the law enforcement officers in the various countries? How much importance will the Codes of Hygienic Practice have in the coming years? Uncertainties like these are not in the interest of food safety.

Another problem area is with the establishment of microbiological criteria for pathogens in foods. These should be based on Minimal Infective Doses, which in turn should take into account the type of food, the type and virulence of the bacteria and the sensitivity of the consumer group. ILSI Europe has made some recommendations on this subject: however, even if they are followed, the "zero tolerance" concept is likely to remain with us for a few years.

Once the criteria are established, the process of estimating actual risk raises further questions. *V. vulnificus*, *A. hydrophila* and *L. monocytogenes* appear to pose a higher risk to "susceptible" people than to "normal" ones. This finding suggests that in the coming years, it may be necessary to categorize foods as being for "high risk" or "low risk" groups. Is it realistic and realizable to have a policy that all foods should be safe for everyone, or should a well informed consumer be allowed to make his own choice? The problem is complicated by the fact that an increasing percentage of the population is becoming more sensitive to pathogens or potential, "opportunistic" pathogens.

Food safety issues also affect trade. Since it is not realistic to expect all foods on the market to be without potential pathogens, will the "safety" of imported foods not be used to hamper free trade? Since "safety" is not precisely defined, it may be difficult to prove that under good manufacturing, commercialization and preparation practices the food is microbiologically safe. We need better definitions of what is acceptable and what is not acceptable.

As Europe changes, so do European consumers. Their expectations are becoming progressively higher: they demand freshness, exotic tastes, assurances that the producer has respected the environment and, above all, unquestionable safety of all foods on the market. In responding to these demands, the food industry faces many new problems. Changing eating habits may cause foodborne diseases. For example, meeting the consumers' demands for freshness may mean less severe processing and more reliance on chilling conditions. Production of exotic foods may require importation of raw materials of uncertain origin: attempts to adapt traditional production methods for these foods to local conditions may also have disastrous results.

In conclusion, the prevention of foodborne diseases in an increasingly complex society is the responsibility of both regulators and consumers. Taking risks cannot be avoided, but we must learn to approach them in a rational way.

MICROBIAL CONCERNS OF THE NORTH AND SOUTH AMERICAN COUNTRIES AND SCIENTIFIC IMPLICATIONS FOR HARMONIZING FREE TRADE

Lester M. Crawford, Executive Vice President, Scientific Affairs, National Food Processors Association, 1401 New York Avenue, N. W., Washington, DC 20005

Discussion will center on three points: 1) an international perspective on the significance of foodborne microbial pathogens in the global trade in foodstuffs; 2) sound science as a basis for resolving regional and worldwide trade disputes; 3) the relevance of HACCP and ISO 9000 in resolving trade barriers based on microbial concerns. The dispute resolution mechanisms within the Canada-U.S. Free Trade Agreement, the North American Free Trade Agreement, and the General Agreement on Tariffs and Trade will be detailed and considered.

FOOD MICROBIOLOGICAL CRITERIA OF THE SOUTH AMERICAN COUNTRIES

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Microbiological criteria for food are important for public health and the consumers, as well as to facilitate the international trade. Their establishment requires of uniform systems for food analysis and consensus on the methodology to be applied internationally.

Codex Alimentarius, FAO, WHO, ICMSF, have greatly contributed providing useful methodology to be applied by developing countries. Most of the South American countries are members of the Codex Alimentarius.

Each country has its own National Commission for Standardization of food and several Ministeries are usually involved in the establishment of these microbiological criteria. The number of approved standards goes from 10 to 80 and, in all the countries the number of analysis to be performed is practically the same (SPC, coliforms, yeast and mold, *Cl. perfringens*, *B. cereus*, *S. aureus*, and *Salmonella*). However does not exist harmonization concerning sampling plans and the expression of results. On this respect, there are three groups among the twelve Latin American countries: 1) those who apply the ICMSF sampling plans, 2) those who are beginning the implementation of sampling plans and 3) those that do not apply sampling plans yet. Due to the fact that most of the South American countries are food exporters, they must fulfill as well with all the international specifications required by the importing countries. The detention or rejection of a shipment cause a significant loss for the exporting countries.

Meat, poultry and eggs, are the most frequent vehicles of *Salmonella*, for this reason the health agencies in each country have improved and increased their control and surveillance over foodstuffs, especially those entering international trade. But a new concern related to emergent pathogens has arised. Appropriate steps should be taken to implement rapid methods of detection for these microorganisms in developing countries. It is a need that Latin American countries receive more assistance from international agencies, in order to improve food quality, harmonize food requirements and apply adequate food controls.

The emergent concept of the HACCP, a preventive control system, rational and systematic, with a better cost-benefit is of prime importance for developing countries.

HACCP seems to be the best strategy to make the food supply safer.

MICROBIAL CONCERNS OF THE PACIFIC RIM COUNTRIES AND SCIENTIFIC IMPLICATIONS FOR HARMONIZING FREE TRADE

Michael Eyles, Product Manager, CSIRO Food Research Lab, P.O. Box 52 North Ryde, New South Wales 2113, Australia

The development of effective and realistic methods for assuring the microbiological safety of foods in international trade is of considerable importance to countries like Australia and New Zealand, which export substantial amounts of food. HACCP and the ISO 9000 series of standards are viewed as important aids to improving the efficiency of processes, assuring the microbiological quality of foods, and achieving greater harmonization of international and domestic regulatory requirements. They have been accepted as the basis for quality systems by many food processors in Australia and New Zealand, particularly in the dairy industry, where major companies have gained or are seeking third-party certification to ISO 9000 standards. Many regulatory authorities responsible for the safety of foods are also basing their requirements on HACCP and ISO 9000. However these systems have limitations. Mechanisms for preventative quality assurance for some unprocessed raw commodities are not well developed; the control of viruses and marine toxins in fishery products provides examples that are relevant to this part of the world. Quality systems based on HACCP and ISO 9000 can be used successfully to assure the microbiological quality of foods in international trade only if there is agreement on the microbiological quality required. There is a need for better methodologies for assessing the risks associated with pathogens in foods and achieving scientific consensus on microbiological hazards.

SAFETY AND QUALITY MANAGEMENT THROUGH HACCP AND ISO 9000

Michael F. Stringer, Head of Microbiology Department, Campden Food and Drink Research Association, Chipping Campden, Gloucestershire, GL55 6LD, United Kingdom

In the European food and drink industry, much emphasis is being placed on the potential commercial benefits of improved systems for quality management.

With the necessity to harmonize national legislation throughout Europe and the requirement for European Regulations and Directives, increasingly food companies are considering the scope and relevance of a number of accreditation and certification systems related to quality.

HACCP, an acronym for Hazard Analysis Critical Control Point, is a tool that enables management to introduce and maintain a cost-effective, ongoing food safety programme. It is now accepted internationally as the most appropriate system for assessing and managing the safety of products and as a product concept is often referenced in legislation and codes of manufacturing practice.

The paper summarizes:

- Specific requirements and approaches of HACCP and ISO 9000 and how they relate.

- Effective implementation.
- Experiences in Europe with particular reference to ISO 9000
- The impact of European legislation on the use of these quality management tools.

TECHNICAL SESSION — ANTIMICROBIALS

ANTIMICROBIAL ACTIVITY OF LACTIC ACID BACTERIA ISOLATED FROM READY-TO-EAT TURKEY PRODUCTS

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Various ready-to-eat turkey products were purchased and their spoilage flora examined to isolate lactobacilli to screen for antimicrobic substances. Selective plating procedures, in addition to standard plate counts, were used to isolate lactic acid bacteria (LAB). The catalase-negative, gram-positive bacilli underwent carbohydrate fermentation tests to determine their genera and species. In order to screen for the production of antibiotic substances, the flip-plate method of Kekessy and Piguet (1970) was used. Several strains of lactobacilli were found to be inhibitory to the gram-positive flora of these products. Spent culture media from the inhibitory LAB were neutralized, filter-sterilized, and incorporated into agar for spot-plate testing. At pH 7, activity was abolished. However, at pH 6, media from one strain of *L. sake* and one strain of *L. curvatus* showed narrow spectrum inhibition of three strains of lactobacilli. Media obtained from an unidentified *Pediococcus* sp. inhibited one Streptococcus and one strain of *L. coryneformis*.

EFFICACY OF USING ANTAGONISTIC MICROORGANISMS TO INHIBIT PSYCHROTROPHIC PATHOGENS IN REFRIGERATED, COOKED POULTRY.

Y.-Y. Hao*, R.E. Brackett and M.P. Doyle, Food Safety and Quality Enhancement Laboratory, University of Georgia, Food Science and Technology Department, Griffin, GA 30223-1797

The ability of 7 antagonistic bacteria to inhibit growth of *Aeromonas hydrophila* K144 (AH) and *Listeria monocytogenes* Scott A (LM) in refrigerated, cooked chicken breast was studied. Cooked chicken breasts were inoculated to result in populations of 10^1 or 10^5 cfu of AH and LM/g. Breasts were then inoculated with individual antagonistic bacteria to result in 10^3 cfu of antagonists/g. Treated samples were packaged and incubated at 5° or 15° C for 2 or 1 weeks, respectively. Populations of the two pathogens were determined periodically using starch ampicillin agar (AH) or modified Oxford agar (LM). Antagonistic bacteria differed significantly in their ability to inhibit growth of the two pathogens. In general, *Carnobacterium piscicola* LV17, *Lactobacillus bavaricus* MN, *Leuconostoc paramesenteroides* OX, and *Lactococcus lactis* 11454 were most effective at inhibiting growth of AH or LM. However, initial populations of LM or AH and incubation temperature affected the efficacy of antagonists to inhibit the pathogens. After 1 week incubation at 15° C, breasts inoculated to contain 10^1 cfu pathogens/g and not treated with antagonistic bacteria contained as much as 1.5 and 2 $\log_{10}$/g more AH and LM, respectively, than treated breasts. Antagonistic bacteria tested that were less effective at inhibiting growth of AH and LM included *Lactobacillus sake* Lb 706, and *Pediococcus acidilactici* strains M and PAC 1.0.

THE ROLE OF METABOLIC INTERMEDIATES IN THE INHIBITION OF *SALMONELLA ENTERITIDIS* BY A *VEILLONELLA* SPECIES

Arthur Hinton, Jr.*, Assistant Professor, Michael E. Hume, and John R. DeLoach, Auburn University, Department of Botany and Microbiology, Auburn, AL 36849-5407

A veillonellae bacterium was isolated from the cecal contents of adult chickens. The *Veillonella* was grown on an agar medium supplemented with either tartrate, lactate, pyruvate, malate, fumarate, or succinate and on a control agar medium that contained no added supplements. *Veillonella* growth was overlaid with fresh agar media, and *S. enteritidis* was spread on the surface of the overlay. *Veillonella* inhibited the growth of *S. enteritidis* on media supplemented with tartrate, lactate, malate, fumarate, or succinate; but growth of *S. enteritidis* was not inhibited on control media or media supplemented with pyruvate. The pH and the concentration of acetic and propionic acid of control and supplemented broth media inoculated with *Veillonella* were also measured. Inhibition of the growth of *S. enteritidis* was related to the ability of *Veillonella* to convert the metabolic intermediates into inhibitory concentrations of acetic and propionic acids.

INHIBITION OF *LISTERIA MONOCYTOGENES* AND OTHER BACTERIA BY SODIUM DIACETATE

Lakshmi Addala and Leora A. Shelef*, Department of Nutrition and Food Science, Wayne State University, Detroit, MI 48202

Growth and survival patterns of *L. monocytogenes* strain Scott A in media containing acetic acid, sodium acetate, combination of the two, and sodium diacetate ($CH_3COOH.CH_3COONa$) were studied, and effects of acetate concentrations, media pH, and temperature evaluated. Sodium diacetate was the most effective inhibitor in the pH range of 5.0-6.0. Minimum inhibitory concentrations in BHI broth (pH 5.4-5.8) decreased with decrease in temperature, from 35 and 32 mM at 35° and 20°, respectively, to 25 mM at 5°C. Addition of sodium diacetate (21 and 28 mM; 0.3 and 0.4%) to ground beef suppressed total aerobic counts during refrigerated storage. Although the meat pH decreased to 5.0-5.2 by the addition of the compound, a significant part of the antimicrobial effect was attributed to the diacetate. Sodium diacetate suppressed growth of two additional *L. monocytogenes* strains and gram-negative bacteria consisting of *Escherichia coli*, *Pseudomonas fluorescens*, *P. fragi*, *Salmonella enteritidis*, and *Shewanella putrefaciens*, but had no effect on *Yersinia entercolitica*, *Enterococcus faecalis*, *Lactobacillus fermentis* or *Staphylococcus aureus*. The use of sodium diacetate is recommended to control growth of listeriae in prepared foods, particularly in meat, poultry and fish products.

ANTIMICROBIAL EFFECTS OF TRISODIUM PHOSPHATE AGAINST BACTERIA ATTACHED TO BEEF TISSUE

J.S. Dickson*, C.G. Nettles and G.R. Siragusa, U.S. Department of Agriculture, ARS, Roman L. Hruska U.S. Meat Animal Research Center, P. O. Box 166, Clay Center, NE 68933

Beef tissue was inoculated with *Salmonella typhimurium*, *Listeria monocytogenes* and *Escherichia coli* O157:H7. The tissue was sanitized with trisodium phosphate at 25°C, 40°C and 55°C with contact times of up to three minutes, at a minimum concentration of 8%. Reductions in bacterial populations of 1 to 1.5 $\log_{10}$ cycles were seen on lean tissue with the Gram-negative pathogens, although less reduction in population was seen with *L. monocytogenes*. Greater reductions in bacterial populations were observed on adipose tissue, with maximum reductions of 2 to 2.5 $\log_{10}$ cycles and 1 to 1.5 $\log_{10}$ cycles for the Gram negative and Gram positive pathogens, respectively. Typically greater reductions in bacterial populations were seen as the temperature of the trisodium phosphate solution increased. Beef tissue was inoculated with *E. coli* ATCC 25922 and sanitized with 8% trisodium phosphate using a model carcass washing system. Population reductions on lean tissue were comparable to those observed in the laboratory with *E. coli* O157:H7. However, greater reductions were observed on adipose tissue from the model system, suggesting that the physical washing procedure may have contributed to the reduction in the bacterial population.

ANTILISTERIAL ACTIVITIES OF LACTIC ACID SALTS IN SAUSAGE AND THE RELATIONSHIP TO PH AND WATER ACTIVITY

Leora A. Shelef, Professor, Department of Nutrition and Food Science, 3009 Science Hall, Wayne State University, Detroit, MI 48202

Sodium, potassium, and calcium salts of lactic acid are approved GRAS additives in foods to enhance flavor, increase water holding capacity and for other purposes. Antimicrobial activities, of the Na salt in particular, have been reported, and evidence is accumulating for antilisterial activity of the salt in meat products. Additions of 4% Na or K lactate to cooked pork liver sausage containing 2% NaCl followed by heat sterilization and artificial inoculation with *L. monocytogenes* strain Scott A suppressed cell growth for 10 days at 20°C, and growth suppression was enhanced at 5°C during storage for 50 days. Growth was inhibited at either storage temperature by the addition of 3% of Ca lactate, and cell numbers declined by ~ one log cycle. Water activity of the sausage was 0.965, and levels were reduced by ≤0.01 units with additions of the lactates. The product pH was 6.04, and levels increased by less than 0.05 units with additions of the Na or K salts, and decreased by 0.6 units with the Ca salt. Analysis of the data showed listeriostatic effects in the product at combinations of mean water activity of 0.951 and pH of 6.08 (Na and K lactate), and of mean water activity of 0.968 and pH of 5.52 (Ca lactate). These water activity thresholds are somewhat higher than those reported for *L. monocytogenes* in humectant-adjusted broth.

TECHNICAL SESSION — RISK ASSESSMENT AND EDUCATION

ANALYSIS OF *LISTERIA* RISK MANAGEMENT FOR FOOD PROCESSORS

Deborah Amaral and Lee Ann Jaykus*, Ph.D. Candidate, Department of Environmental Sciences, University of North Carolina, Chapel Hill, NC 27599

An economic model of the impact of a product testing strategy was developed for *Listeria*, and implemented for a hypothetical product testing scenario. The model includes a representation of the direct and indirect costs of an outbreak of Listeria, product recall, and the cost of testing and of destruction of product. Probabilistic methods are used to represent uncertainty about these costs, as well as the likelihood of product contamination, disease transmission, and the quality of the testing methods. The most sensitive variables in the model were the probability of disease in the exposed population, and the number exposed, and it is recommended that future emphasis be placed on obtaining a more accurate characterization of both phenomena. This approach is applicable to other microbial contaminants and other potential management options, and can also provide useful insights about incentives to reduce risks, when applied from a regulatory perspective.

THE IMPACT OF EMPLOYEE FOOD SANITATION KNOWLEDGE AND HANDLING PRACTICES ON SUPERMARKET DELI PROFITABILITY

Gene A. Thomas, Robert B. Gravani*, Professor, Edward W. McLaughlin and H.T. Lawless, Cornell University, Department of Food Science, 8A Stocking Hall, Ithaca, NY 14853

A comprehensive 47-question survey instrument was developed and administered to nearly 800 deli workers in 58 stores of an eastern supermarket chain to determine if food sanitation and safety knowledge correlated directly with improved food handling practices and department profitability.

Deli employee mean knowledge index scores increased with education, on-the-job-training and job title. Scores increased with age (up to 45 years) and deli experience (up to 10 years) and then declined.

Using the chi-square statistical test, a correlation between employee mean knowledge index scores and deli profitability was shown. Cross tabulations of key food safety terms and concepts showed no association between knowledge and practice for many respondents.

EDUCATING FIFTH GRADERS ABOUT FOOD SAFETY THROUGH THE USE OF A VIDEO

Gloria I. Swick, M.S.A., R.S., Public Health Sanitarian, Columbus Health Department, 181 S. Washington Boulevard, Columbus, OH 43215

Children should be taught proper food handling procedures in the schools. Little research has been done to date and few appropriate, effective visual aids are available.

Exploration of the level of knowledge and comprehension of fifth grade children about food protection was accomplished by administering a pretest. Based on the knowledge they possessed, an instructional video was produced to teach basic concepts of proper food handling such as handwashing and temperature control of food. The video showed children cooking in a kitchen and included rap music and dancing.

Immediately after viewing the video, the original group of fifth graders were retested in order to measure educational growth. The post test showed 71% of the student's scores increased from the scores of the pretest as a result of viewing the video.

RELIABILITY OF POP-UP TIMERS IN TURKEYS

Marilyn B. Lee, Professor, School of Environmental Health, Ryerson Polytechnical Institute, Toronto, Ontario, Canada M5B 2K3

The study was undertaken to assess the reliability of pop-up timers (3M Company) in assessing turkey doneness. Sixteen turkeys, 8 stuffed and 8 unstuffed, weighing 10 -25 lbs., were cooked until the timers "popped". Temperatures of meat at the pop-up timer point of insertion were recorded as well as the minimum internal temperatures of the centre of the meat or stuffing. Results revealed that birds weighing 25 lbs., both stuffed and unstuffed and stuffed birds weighing 20 lbs. had timers popping when the timer reached 181-185°F (83-85°C). Yet, the minimum internal temperature of the bird was 132-139°F (55.6-59.6°C). These temperatures would not consistently be sufficient to ensure destruction of salmonellae. Pop-up timers may prove more satisfactory in unstuffed birds weighing 20 lbs. or less, and stuffed birds weighing 15 lbs. or less. The best method for assessing turkey doneness remains the meat thermometer probe.

FOOD SANITATION IN THE ICE AGE

Charles W. Felix, M.P.H., Charles Felix Associates, P.O. Box 1581, Leesburg, VA 22075

Ice has become a staple of society's casual, mobile lifestyle. Americans consume more ice than they do bread—about 2.2 pounds per person per day. Concern over the sanitary quality of packaged ice was raised in 1987 when an iceborne viral outbreak in Pennsylvania and Delaware caused 5,000 illnesses. Since that time the industry and regulatory agencies in the states have taken steps to assure that ice is manufactured and packaged in a sanitary manner. Principles of HACCP are relatively unknown to ice manufacturers and in some instances Good Manufacturing Practices are not followed. A survey taken in 1993 to ascertain the status of ice regulation in each of the 50 states and 10 Canadian provinces will be reported. Critical control points for prevention of illnesses from contaminated ice need to be identified and implemented.

GENERAL SESSION — COMMUNICATING FOOD SAFETY IN THE NEWS

PUTTING TOGETHER A FOOD SAFETY STORY

Kay Flowers, WXIA TV, Health Reporter, 11 Alive News, 1611 W. Peachtree Street, NE, Atlanta, GA 30309

With the deadly outbreak of *E. coli* in the western United States, food safety concerns have been in the news and on the minds of many people. How do reporters, especially television reporters, put together the reports they present on these issues? Where do they get their information, and who are their sources? Who decides how much time to give each report in a newscast and what importance to place on it? I will outline the process by which a food safety story is researched, produced and aired; and answer any questions the attendees may have.

IMPACT OF A NEWS STORY ON THE FOOD INDUSTRY

Lester Crawford, National Food Processors Association, 1401 New York Avenue, NW, Washington, DC 20005

The discussion will concern the "Do's and Don'ts" of food safety communication. These relate to the two communication modes: the proactive and the crisis modes. Proactive communication should involve a calm, well-ordered presentation of the facts featuring a definition of the inherent risk, a frank statement explaining what your organization is doing about the problem, and a clear statement of what consumers can do to protect themselves. Crisis communication must proceed as soon as a crisis is identified but it must not deal in unverified facts and/or speculation. Instead, assurance should be given that updates will follow and conclusions will be given as soon as possible. Above all, be hones and convey any and all ways that consumers can protect themselves.

CRITERIA FOR A GOOD NEWS ITEM

Scott Bronstein, Atlanta Journal and Constitution, Box 4689, Atlanta, GA 30302

A discussion of what constitutes news and news reports and how reporters try and view scientific information.

DO'S AND DON'TS FOR INDUSTRIAL SPOKESPERSONS

Michael C. Robach, Director, Quality Assurance, Continental Grain Company, 3700 Crestwood Parkway, Suite 1000, Duluth, GA 30136

In this day and age of intense media coverage and investigative reporting, industrial spokespersons must be able to provide accurate, thoughtful information in a timely manner. Requests for information should be handled promptly, however statements should be made after taking time to think. Answers to leading questions and "off the cuff" statements should be avoided. Answers should be forthright and truthful and the spokesperson should not be evasive. Always be accurate, never estimate data or forecast a result, deal with the facts in the proper context and above all, use simple common sense.

PUBLIC EDUCATION TO ENHANCE FOOD SAFETY

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

Communicating the risks and benefits of food safety issues to the public is a significant challenge. Scientists frequently provide television, radio and print journalists with highly technical information that ultimately fails to help them or their publics understand the scientific facts associated with the issue. Often, wordy responses filled with technical jargon confuse consumers and prevent them from making informed decisions about issues of concern.

Through the use of case study examples, this presentation will focus on techniques that can be used to improve the quality and understandability of food safety messages that are presented to the public. A variety of strategies that can be used to improve communication of the risks and benefits of food safety issues will be highlighted and discussed.

ILSI-SPONSORED RESEARCH UPDATE

Sponsored by the International Life Sciences Institute

ESCHERICHIA COLI O157:H7 DIARRHEA IN THE UNITED STATES: A MULTICENTER SURVEILLANCE PROJECT

Patricia M. Griffin*, A. A. Ries, K. D. Greene and the *Escherichia coli* O157:H7 Study Group, Epidemiology Section, Enteric Diseases Branch, National Center for Infectious Diseases, National Centers for Disease Control, 1600 Clifton Road, Building 1-54428, M/S CO9, Atlanta, GA 30333

Escherichia coli O157:H7 causes nonbloody and bloody diarrhea and hemolytic uremic syndrome (HUS). Because no national surveillance system exists, the magnitude of *E. coli* O157:H7 infections in the U.S. is not known. To define its epidemiology, 10 study hospitals throughout the U.S. cultured all 26,239 submitted stool specimens for *E. coli* O157:H7, using sorbitol-MacConkey medium, and for routine pathogens for 2 years, and collected epidemiologic and clinical data.

E. coli O157:H7 was isolated from 99 (0.4%) specimens, compared with *Campylobacter*, 2.2%; *Salmonella*, 1.8%; and *Shigella* 1.0%. *E. coli* O157:H7 was isolated from 7.8% of specimens with visible blood, more than *Campylobacter* (5.7%), *Salmonella* (3.4%), or *Shigella* (3.6%). Only 68% of *E. coli* O157:H7 isolates were from specimens with visible blood. *E. coli* O157:H7 was isolated more frequently at hospitals in northern and western states than in southern states ($p<0.01$). It was the second most frequently isolated bacterial enteric organism in one hospital, and the third most frequent in three. Sixty-four percent of *E. coli* O157:H7 isolations occurred between June and September. The age groups with the highest number of *E. coli* O157:H7 isolates were 0-9 years (17) and ≥60 years (15). Fifty percent of patients were hospitalized, 5% had HUS, and none died.

E. coli O157:H7 is a frequent cause of bloody and nonbloody diarrhea in the United States. Clinicians should include *E. coli* O157:H7 in their differential diagnosis of diarrhea, especially bloody diarrhea. At a minimum, laboratories should culture all stools from persons with bloody diarrhea for *E. coli* O157:H7.

ESTABLISHMENT OF A BOVINE SURVEILLANCE PROGRAM FOR *E. COLI* O157:H7 IN WASHINGTON STATE

Dale Hancock, Field Disease Investigation Unit, Department of Veterinary Clinical Medicine and Surgery, Washington State University, Pullman, WA 99164-6610

Serious human illnesses, including hemorrhagic colitis and the hemolytic uremic syndrome, are associated with *E. coli* O157:H7. Cattle are a reservoir of this agent as shown by fecal direct culture and by epidemiologic associations in outbreaks of *E. coli* O157:H7 related disease. The goal of this study was to investigate the bovine reservoir of *E. coli* O157:H7 in Washington state. *E. coli* O157:H7 was found in 10 of 3750 (0.28%) fecal samples from dairy cattle in 5 of 60 herds (8.3%). Small herd size, use of computerized feeders, and irrigation of pastures with manure slurry were significantly associates with the occurrence of *E. coli* O157:H7 on dairy farms. Feeding of whole cottonseed was negatively associated with the occurrence of *E. coli* O157:H7. Easily obtainable pooled samples from dairy herds (fecal slurry samples, bulk milk samples, and milk filters) were uniformly negative for *E. coli*O157:H7. *E. coli*O157:H7 was also isolated from 10 of 1412 (0.71%) fecal samples from beef cattle in four of 25 (16%) cow/calf herds. Feedlot beef cattle had a prevalence of *E. coli* O157:H7 shedding similar to that of dairy cattle (2/600, 0.33%). Results of this study indicate that beef cattle may be a larger reservoir of *E. coli* O157:H7 than previously thought. The identification of cattle management practices associated with colonization of cattle by *E. coli* O157:H7 raises the possibility that human *E. coli* O157:H7 exposure may be reduced by management procedures affecting the bovine reservoir.

INSERTION SEQUENCE FINGERPRINTING: A NEW SUBTYPING SYSTEM FOR *E. COLI* O157:H7 STRAINS

Thomas Whittam, Department of Biology, 208 Erwin W. Mueller Laboratory, The Pennsylvania State University, University Park, PA 16802

Insertion sequences (IS) are small segments of the chromosomes of bacteria cells that have the ability to copy and transpose from one place on the chromosome to another. In populations of *Escherichia coli* exist in 5-50 copies per cell. Previous studies have shown that *E. coli* strains that are indistinguishable by many biochemical tests differ in both the number and positions of different IS elements. This means that restriction digests of genomic DNA and subsequent Southern hybridizations with IS-specific probes can reveal distinctive patterns or "IS fingerprints" for *E. coli* strains. We tested specific DNA probes for 3 different IS elements, and discovered two elements (IS30 and IS3) that were variable, and thus gave different fingerprints, among 33 O157:H7 strains that were otherwise similar in biochemical tests. With these IS fingerprints, we have been able to determine that certain IS subtypes have spread into several countries, and some occur both in humans and cows, an observation supporting the hypothesis that bovine herds are reservoirs for O157:H7 strains. Further study of *E. coli* O157:H7 strains with IS fingerprinting may be useful for elucidating how these bacteria spread from one place to another and the routes by which they are transmitted from animals to humans.

USE OF *IN VITRO* PRIMER-DIRECTED ENZYMATIC AMPLIFICATION OF DNA FOR RAPID DETECTION OF *LISTERIA MONOCYTOGENES*: STUDIES WITH FOOD SAMPLES

Richard T. Ellison III, M.D., Division of Infectious Diseases and Immunology, University of Massachusetts Medical Center, S6-753, 55 Lake Avenue North, Worcester, MA 10655

A rapid sensitive system for screening foods and clinical samples for *Listeria monocytogenes* will be of great value in the prevention of foodborne listeriosis. We have studied the utility of the polymerase chain reaction technique (PCR) for rapid *Listeria* detection. DNA primers to the listeriolysin O gene were selected that define a 606 base-pair sequence, and amplified listerial DNA products are detectable by agarose gel electrophoresis or dot-blot analysis with a [^{32}P]-labeled internal probe. In tests with differing bacterial strains excellent sensitivity has been observed; 95 of 95 *L. monocytogenes* strains were positively identified. The assay also has high specificity, negative results have been obtained with 12 of 12 non-*monocytogenes Listeria* strains and 12 of 12 purified non-*Listeria* strains. The assay can be directly applied to milk samples with a sensitivity of 10^1 cfu/ml. Additionally, it can be combined with a preamplification broth incubation to enhance sensitivity and diminish false-positive results due to killed *Listeria*. The assay requires approximately 28 hours, and has achievable levels of sensitivity in spiked food samples of 10^{-1} cfu/ml in milk; 10^{-1} cfu/g in processed meat; 10^0 cfu/g with radishes, and 10^3 cfu/g in cheese without the use of radioactive probes.

DEVELOPMENT OF DNA PROBES SPECIFIC FOR VIRULENT *LISTERIA* BY AMPLIFICATION OF VIRULENCE-RELATED GENES OF *LISTERIA MONOCYTOGENES*

Sophia Kathariou, Department of Microbiology, Synder Hall 207, 2538 The Mall, University of Hawaii/Manoa, Honolulu, HI 96882

The goal of our work is to identify and characterize genes of *Listeria monocytogenes* which are involved in the expression of specific virulence-related characteristics of the bacteria. One such gene is important for the ability of *Listeria* to enter (invade) mammalian cells in culture. We have generated a transposon mutant which is deficient in invasion of mammalian cells as well as in virulence. We have used a modification of the Polymerase Chain Reaction (PCR) to isolate a *Listeria* DNA fragment flanking one side of the transposon in the noninvasive mutant. On the basis of the nucleotide sequence of this DNA fragment we can generate primers which can be used for PCR-based detection of the bacteria.

Interestingly, the noninvasive mutant failed to be recognized by the serotype 4b-specific monoclonal antibody C74.22, suggesting that the transposon insertion may have caused loss or alteration of a serotype 4b-specific antigen on the surface of the bacteria. We are using C74.22 and other serotype 4b-specific monoclonal antibodies which we have generated in order to identify genes involved in the expression of the corresponding antigens. Such genes may be suitable for generation of DNA probes specific

for serotype 4b *Listeria*. Tools for rapid and accurate detection of such strains may be valuable, since this serotype of *Listeria monocytogenes* has been implicated in many sporadic cases of listeriosis as well as in all major food-related outbreaks of the disease.

MICROBIAL ECOLOGY OF *LISTERIA MONOCYTOGENES* BIOFILMS ASSOCIATED WITH THE FOOD PROCESSING PLANT ENVIRONMENT

Joseph F. Frank, Department of Food Science and Technology, University of Georgia, Athens, GA 30602

The growth of *Listeria monocytogenes* on surfaces in food processing plants may present an acceptable public health risk if foods susceptible to post-process contamination are being processed. Microorganisms growing on surfaces produce a combination of cells and extracellular metabolites called a biofilm. Biofilms provide protection from sanitizing agents and therefore must be removed by cleaning procedures if effective sanitization is to be achieved. *L. monocytogenes* formed biofilms on stainless steel, teflon, nylon, and floor sealant at 10 and 21°C. Biofilms were formed on these surfaces in both minimal and complex media, except for floor sealant which only supported biofilm growth in a complex medium. The ability of *L. monocytogenes* to attach to stainless steel was decreased by growth of the cells in a medium containing digested protein, whereas biofilm growth of *L. monocytogenes* was stimulated by the presence of hydrolyzed protein. *L. monocytogenes* attached to stainless steel in the presence of competing microflora. In addition, *L. monocytogenes* was able to survive and grow in multispecies biofilms.

CONTROL OF BACTERIA AND PUBLIC HEALTH SIGNIFICANCE IN FOODS OF ANIMAL ORIGIN SYMPOSIUM

USDA APHIS PRE-HARVEST FOOD SAFETY INITIATIVE/NAHMS SURVEY

Thomas M. Gomez*, Epidemiologist, and Robert V. Tauxe, USDA/APHIS/VS CDC/NCID/DBMD/EDB, 1600 Clifton Road, MS C-09, Atlanta, GA 30333

The recent *Escherichia coli* O157:H7 outbreak in the western United States has refocused national attention on the issues surrounding food safety from the farm to the table. This paper presents the USDA, APHIS, perspective on implementing a National Food Safety Program that focuses on pre-harvest (farm-transport-auction market) activities. An overview and study results from the APHIS National Animal Health Monitoring System (NAHMS), which supports APHIS food safety initiatives, are also presented.

COMPETITIVE EXCLUSION AND POULTRY

Nelson Cox, U. S. Department of Agriculture, ARS, Russell Research Center, Box 5677, Athens, GA 30613

The most effective means currently known to reduce salmonellae colonization of growing chickens is competitive exclusion. We have developed an undefined mucosal competitive exclusion (MCE) culture containing a diversity of flora which successfully diminishes *Salmonella* spp. and *Campylobacter* spp. in commercial scale field trials. Utilizing MCE reduced the incidence of *Salmonella* on fully processed carcasses four fold. Data from another trial indicated that *Campylobacter* numbers associated with carcasses were reduced by two logs. Hatchery acquired salmonellae has been shown to reduce the effectiveness of MCE therefore future field trials will attempt to minimize hatchery contamination.

CONTROL OF BACTERIA OF PUBLIC HEALTH SIGNIFICANCE IN FOODS OF ANIMAL ORIGIN

David M. Theno* and J. O. Reagan, Foodmaker, Inc., 9330 Balboa Avenue, San Diego, CA 92123-1516

The control of bacterial pathogens in foods of animal origin has been and continues to be a scientific area of active research. In foods which will not be heat processed prior to sale to consumers control of bacterial pathogens is best accomplished through HACCP based control system which encompass farm to finished package operations. Thorough cooking is the best method to eliminate bacterial pathogens in ready to eat foods (those that will not be further heat processed) or in raw products which will be prepared for serving (either in the home or in an institutional setting). HACCP based programs are the methods of choice for ensuring that all foods of animal origin are free of bacterial pathogens and safe for consumption.

USE OF BACTERIOCINS AS BIOCONTROL AGENTS IN FOOD — AN UPDATE

Gregory R. Siragusa, USDA/ARS, RLH, U. S. Meat Animal Research Center, P. O. Box 166, Clay Center, NE 68933

Research in the area of bacteriocins has increased dramatically in the last decade. This presentation will summarize applications of bacteriocins to problems of microbial food safety. Also covered will be data on newly described bacteriocins; their discovery and potential applications. Emphasis will be on means of enhancing efficacy of currently approved compounds and will include a discussion of areas of potentially fruitful research.

FSIS' NATIONWIDE MICROBIOLOGICAL BASELINE DATA COLLECTION PROGRAMS

Ann Marie McNamara, Director, Microbiology Division, Food Safety and Inspection Service, U. S. Department of Agriculture, 300 12th Street, S.W., Room 410 Annex, Washington, DC 20250

The Food Safety and Inspection Service of the United States Department of Agriculture has proposed a series of Nationwide Microbiological Baseline Data Collection Programs. These programs were designed to collect human foodborne pathogen data on a variety of animal species slaughtered under Federal inspection. Using the Nationwide Beef Microbiological Baseline Data Collection Program as a model, the concept, design, and implementation of these programs will be discussed.

ESCHERICHIA COLI O157:H7: HISTORICAL, EPIDEMIOLOGICAL AND CLINICAL ASPECTS

Phillip I. Tarr, M.D., Assistant Professor, Gastroenterology and Infectious Diseases, Children's Hospital and Medical Center, 4800 Sand Point Way, N.E., P. O. Box C5371, Seattle, WA 98105

Escherichia coli O157:H7 has been well studied in the Pacific Northwest since its initial description a decade ago. This organism is recovered almost as frequently in childhood as *Campylobacter* and *Salmonella* in laboratories which routinely screen for its presence. *E. coli* O157:H7 causes a spectrum of illness, but most commonly is associated with painful bloody diarrhea (hemorrhagic colitis). It is the most common, and possibly the exclusive, precipitant of hemolytic uremic syndrome in the Pacific Northwest. Data suggest that the incidence of infections caused by this pathogen have increased during the past two decades. Clinical and investigative issues revolve around appropriate treatment of infected patients to ameliorate the hemorrhagic colitis and avoid hemolytic uremic syndrome, and prevention of this pathogen from entering the food supply.

VIRAL FOODBORNE DISEASE SYMPOSIUM

VIRAL FOODBORNE DISEASE AGENTS OF CONCERN

Dean O. Cliver, Professor, Food Research Institute, University of Wisconsin-Madison, 1925 Willow Drive, Madison, WI 53706

Viruses transmitted to humans via foods generally emanate from the human intestines. In the U.S., Norwalk virus ranked #5, hepatitis A virus #6, and "other viruses" (principally rotavirus) #10 among the top 10 causes of foodborne disease during 1983-1987. Molluscs are the most frequently reported vehicles, but any food handled by humans may transmit human enteric viruses. Some fruit and vegetable vehicles may have been contaminated in the field before or during harvesting. Viruses in foods may be inactivated before the food is eaten, and thus not cause infection. Increasingly sensitive detection methods, largely based on "molecular" techniques, are becoming available for these viruses but are not applicable to monitoring foods on a routine basis.

THE EPIDEMIOLOGY OF VIRAL FOODBORNE DISEASE

Dean O. Cliver, Professor, Food Research Institute, University of Wisconsin-Madison, 1925 Willow Drive, Madison, WI 53706

Virus transmission via foods begins with fecal shedding of viruses by humans. Foodborne viruses infect perorally: these same agents have alternate fecal-oral routes, including person-to-person transmission and the water vehicle. No zoonotic viruses are transmitted via foods in North America.

Viruses rank high among foodborne disease agents in the U.S., even though observation, diagnosis, and reporting of foodborne viral disease are inefficient. Risk assessment in developed countries considers viral infection rates and personal hygiene of food handlers, as well as the opportunities for contamination of shellfish and other foods by untreated sewage. Licensing of a vaccine against hepatitis A that could be administered to food handlers in North America would provide an important means of preventing foodborne viral disease.

NORWALK VIRUS GASTROENTERITIS: DIAGNOSIS AND EPIDEMIOLOGY

Christine L. Moe, Ph.D., Research Assistant Professor, Department of Epidemiology, University of North Carolina at Chapel Hill

Norwalk (NW) and other small round structured viruses (SRSV) are recognized as important etiologic agents of acute, nonbacterial gastroenteritis and have been implicated in numerous foodborne and waterborne outbreaks. Investigations of SRSV outbreaks and the characterization of this group of viruses have been limited by the lack of adequate laboratory techniques to detect these agents. Between 1983 and 1988, 10 outbreaks involving a total of 1164 cases of foodborne NW gastroenteritis were reported to CDC. However, it is estimated that NW and NW-like viruses may be responsible for 42% of non-bacterial gastroenteritis outbreaks in the US. Diagnosis of NW infection has relied on the observation of a 27 nm small round structured virus by electron microscopy or on demonstration of seroconversion by an enzyme immunoassay using human reagents. The cloning and sequencing of the NW virus genome in 1990 and the recent expression of NW virus capsid protein have catalyzed progress in the detection of NW antigen and antibodies and in the study of NW epidemiology. We used reverse transcription-polymerase chain reaction (RT-PCR) to screen 144 fecal specimens from outbreaks of gastroenteritis and detected NW or NW-related viruses in 39% of the fecal specimens and in 15 of 19 outbreaks of gastroenteritis. Sequence analysis of the PCR products from 11 outbreak strains revealed surprising diversity in the polymerase gene. These results and additional studies of this kind will allow further classification of these viruses at a molecular level and extend our knowledge of the epidemiology and transmission of these agents.

DETECTION METHODS FOR VIRAL AGENTS IN FOODS

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Sensitive and specific detection and assay of low levels of human enteric viruses in food samples via their genomes is now becoming feasible using PCR and similar in-vitro enzymatic amplification techniques, followed by oligonucleotide probing of amplified DNA products. Achieving this goal requires the following essential steps: (i) identification and selection of synthetic oligonucleotide primers and probes for genomic sequences of target viruses and virus groups, (ii) testing of PCR primers and oligoprobes for specificity, selectivity and sensitivity, (iii) further clean-up and concentration of typical food sample concentrates for reliable and efficient amplification and hybridization detection, (iv) evaluation of the methods by application to natural or wild-type viruses in field samples, and (v) confirmation that these methods detect viruses that are likely to be infectious and hence pose a risk to human health.

HEPATITIS A FOODBORNE DISEASE

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Food contaminated with hepatitis A virus (HAV) is well recognized as a vehicle for the transmission of hepatitis A, yet only 5% of reported cases can be attributed to contaminated food or water. In the U.S., the most common risk factor for infection is contact with an HAV infected person. Foods involved in outbreaks are usually eaten raw or partially cooked and have either been contaminated by an infected food handler or contaminated where they were grown. Foods have also been contaminated after cooking by infected food handlers. Nucleic acid detection using the polymerase chain reaction (PCR) coupled with sequence analysis has been used to identify HAV and its potential source in foodborne outbreaks involving shellfish and strawberries. It is not know whether detection of HAV by PCR could be used routinely in certifying shellfish that will be consumed raw as a means of eliminating this source of hepatitis A. Recently, hepatitis A vaccine has been shown to be effective in preventing disease transmission, and may become our most effective tool in preventing HAV infection.

FDA COMPUTER DATA BASE AND REPORTING SYSTEMS SYMPOSIUM

NATIONAL MILK DRUG RESIDUE DATABASE

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FDA and NCIMS have recognized for some time the need for a national drug data base on the scope and extent of drug residue test results of the milk supply. The 1991 NCIMS meeting authorized the development of a national system to compile results of milk industry and regulatory analysis of milk for animal drug residues.

FDA has awarded a contract to establish and operate this database system through an independent third party. State regulatory agencies submit drug residue testing information to this system. In addition to state testing, industry testing is submitted to the state for entry into the system. This system is designed to provide information on the extent of drug residue testing of milk in this country.

THE NATIONAL DRUG RESIDUE MILK MONITORING PROGRAM (NDRMMP)

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The Food and Drug Administration's milk safety programs relies on cooperative efforts with the State regulatory agencies. This effort is formalized in a Memorandum of Understanding between FDA and the National Conference on Interstate Milk Shipments. The FDA with the support of the NCIMS initiated the NDRMMP in February, 1991. The NDRMMP is designed to provide an indication of animal drug residues that may be present in milk and the extent that farmers, distributors and veterinarians comply with the Federal Food, Drug and Cosmetic Act. This is done by randomly selecting samples of raw milk from bulk milk pickup tankers, and analyzing those samples for drug residues in FDA laboratories. All samples have been analyzed for eight sulfonamids, and three tetracyclines. Beginning April 1, 1991, analysis for chloramphenicol was added, and on April 1, 1992 beta-lactam analysis was added. From the inception of the program, approximately 850 samples have been analyzed, from all fifty states and Puerto Rico. One sample was violative (above the safe level) for sulfamethazine, four samples contained very low residues or traces of sulfamethazine, one sample contained a trace of chlortetracycline, and one sample contained a trace of sulfadimethoxine.

FDA PRIME CONNECTION

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FDA Prime Connection is the FDA Center for Food Safety and Applied Nutrition's on-line technical information resource on retail food protection, milk safety and seafood safety. It supports the local, state, and federal regulatory agencies and others who use FDA technical and regulatory recommendations by providing current information which is accessible to users through a computer with a modem through local access numbers or toll-free service.

Program documents, Interstate Milk Shippers and Shellfish Shippers Lists, Foodborne Pathogenic Microorganism and Natural Toxins Reference Book, FDA Fish List and CFR references are some of the technical materials which may be found on FDA Prime Connection. Focus areas on HACCP, Milk Safety, Foodborne Illness, Seafood Safety and other technical areas provide users with the opportunity to network and exchange ideas and information. Currently there are over 2000 registered users representing all 50 states, Canada, Europe and New Zealand on the system.

FDA ELECTRONIC INSPECTION SYSTEM (EIS)

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FDA Electronic Inspection System (EIS) is a software package developed under contract to FDA to allow local, state and federal regulatory agencies to maintain a comprehensive inspection program database. EIS can be easily customized by the food, milk or seafood program to provide the data needed by the program management. Complete ad hoc report generation features and data import/export capabilities make the Office System of EIS a powerful tool for management decision-making.

The EIS Field System allows users to input inspection data into a notebook computer and generate establishment inspection reports on-site. A Technical Reference Library is accessible during the inspection. The EIS will be distributed to regulatory agencies without cost this year.

EVALUATION OF VITAMINS IN MILK

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A problem was identified in a milk processing plant in New England in 1991 regarding the addition of vitamins to milk and milk products. As a follow-up to that problem FDA conducted a survey of the good manufacturing practices in dairy plants on the addition of vitamins.

The survey results show widespread variations in the ability of milk processors to maintain vitamin levels within the prescribed parameters.

FDA milk specialists are conducting GMP information on vitamin addition. The National Conference on Interstate Milk Shipments is addressing the problem.

THE FOOD CODE, 1993 RECOMMENDATIONS OF THE PUBLIC HEALTH SERVICE/FOOD AND DRUG ADMINISTRATION

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The Food and Drug Administration is completing work on the first revision of its model food codes in more than ten years. With an anticipated release later this Fall, the new food code contains a number of modifications which reflect both the changes that have occurred in the retail segment of the food industry and the advances that have been made in science and our understanding of illness related to food. The new code also contains many improvements which will make it easier for both operators and regulators to use.

ECONOMICS OF FOODBORNE DISEASE SYMPOSIUM

COSTS OF BACTERIAL FOODBORNE DISEASE: A REVIEW

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Estimates have been made to determine gastroenteritis of foodborne origin in the United States ranging from 24 to 81 million/year, with the most likely figure being 33 million/year, based on extrapolation of health survey data. Salmonellosis case estimates in the Untied States range between 790,000 - 3,690,000 with a median of 1,920,000. This median is approximately 50 times the number of human isolations and 800 times the number of foodborne cases caused by *Salmonella*. If it is assumed that 2,000,000 cases occur each year, the cost for sporadic cases, outbreak cases, deaths and sequelae amount to $1,853 million or $927/case. The 1,850 hospitalized cases and 425 deaths of listeriosis estimated to occur annually in the United States are also very costly (>$500 million). The costs of all bacterial foodborne diseases range between $6 and 11 billion. Irradiation of poultry has been shown to be economic in reducing costs of illness in Scotland, England, Canada, and the United States.

ECONOMIC LOSSES CAUSED BY FOODBORNE PARASITIC DISEASES: A REVIEW

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The limited data on human illnesses and deaths caused by parasitic foodborne diseases in the United States prevents comprehensive reporting on economic losses. In the literature, the most costly is congenital toxoplasmosis with human illness losses estimated at US $0.4 to 8.8 billion annually. Pork, cats, and lamb are the primary vehicles in the United States. Of the 3 types of preventable costs estimated for congenital toxoplasmosis, income loss (reduced earning of persons born severely or moderately retarded because of fetal infection) is two-thirds of the total cost. The next largest costs are special education and residential care costs. Medical costs, however, comprise a small part of the estimated preventable losses.

Bovin cysticercosis and trichinosis are much less severe diseases than congenital toxoplasmosis, but their total annual costs are nevertheless in the millions of dollars. Potential foodborne parasitic diseases that have not yet been costed include giardiasis, diseases caused by fish parasites, and porcine cysticercosis.

IMPACT OF SHELLFISH-ASSOCIATED VIRAL DISEASES IN THE UNITED STATES

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Clear epidemiological evidence has linked viral gastroenteritis and hepatitis with the consumption of contaminated shellfish and outbreaks continue to occur in the U.S. Hepatitis A virus and enteroviruses have been detected in shellfish harvested from approved waters based on the bacteriological indicator and sanitary surveys. Based on risk assessment models, the risk of infection was 1/100 for a single serving of raw shellfish (60g), for a moderately infectious virus and 1/2 for highly infectious virus from the average levels of viruses detected in these surveys. Annual average capita consumption ranges from 53 g to 250 g and if these virus levels were found in 1% of the shellfish this could result in 24,000 infections per year in which 50% may result in clinical disease. Economic burden based on minimal days of productivity and income losses at $675 per case is estimated at $8 million. Possible controls include warning labels and better classification of harvesting waters using scientific health-based criteria.

HUMAN ILLNESS COSTS ASSOCIATED WITH *SALMONELLA* INFECTIONS IN THE UNITED STATES

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In 1985 an outbreak of antimicrobial-resistant *Salmonella typhimurium* infection in Illinois affected an estimated 360,000 persons. A 1989-1990 prospective follow-up study was conducted to examine the prevalence and epidemiological characteristics of *Salmonella* in selected U.S. counties. This report examines the economic costs to the victims of salmonellosis associated with this large outbreak and with diagnosed *Salmonella* infections reported through national surveillance.

THE VALUE OF A HUMAN LIFE

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What is the value of a human life in the event of a premature death? $200,000? $2.5 million? Is the life of a thirty year old man worth more to society than that of an eighty year old woman? The method selected to determine the value of a human life requires these implicit judgements. While the direct costs of illness are easily understood, the indirect costs associated with morbidity or premature death may have a more decisive influence on the conclusions reached from cost-effectiveness and cost-benefit analyses of public health programs. The most common method for measuring indirect costs is the measure of future production potential or the human capital approach. However, this method has serious drawbacks. It is not theoretically based, has intrinsic distributional biases, and fails to capture all of the costs. Alternatively, the willingness-to-pay approach avoids these problems but is empirically difficult to measure. This paper explores the advantages and drawbacks of these two major methodological approaches to the calculation of the value of a human life and presents innovative applications from recent studies. The paper concludes with a discussion of the new Centers for Disease Control and Prevention recommendations for both the calculation and inclusion of indirect costs in economic evaluations of public health programs.

SEQUELAE OF FOODBORNE DIARRHEIC DISEASE: THE REACTIVE ARTHRITIDES

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Foodborne gastrointestinal pathogens may give rise to pathological problems that are far more serious than the temporary inconvenience of diarrhea. Immunocompetent individuals, after an episode of gastroenteritis triggered by *Campylobacter, Salmonella, Shigella*, or *Yersinia*, may suffer certain chronic joint diseases known as the reactive arthritides (reactive arthritis, Reiter's syndrome or ankylosing spondylitis). About 2% of a population exposed to diarrheic infections develop arthritis. There is a strong familial association which indicates that genetic makeup predisposes individuals to arthritis. The reactive arthritides are often severe and add to the health and economic burdens of afflicted individuals. The roles of human genetics and bacterial pathogens in the reactive arthritides will be discussed.

FOOD SAFETY RESEARCH NETWORKS SYMPOSIUM

INITIATION OF A PREDICTIVE FOOD MICROBIOLOGY NETWORK

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The rise of predictive microbiology and computer modeling as a highly active research area within food microbiology has stimulated a need to exchange ideas and data among a widely diverse group of researchers and interested users worldwide. Researchers at the USDA, ARS, ERRC, Microbial Food Safety Research Unit have been attempting to address this need using several approaches. Initially hosting an international meeting to bring together the microbial modelers for the first time, the ARS scientists are currently developing a directory of interested scientists. The ultimate goals are to develop an international Internet "bulletin board" for the exchange of microbiological food safety and quality information, and identify means for greater international collaboration on large scale predictive microbiology initiatives.

NETWORKING IN THE SOUTHERN EXTENSION RESEARCH ACTIVITY INFORMATION EXCHANGE GROUP

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The Southern Region Extension Research Activity Information Exchange Group (SERA-IEG) including research and extension faculty from all southern states met in May 1992. Participants recognized that improving food safety and quality in the Southern Region requires extensive communication, cooperation, and networking, and formed four working groups. The first group submitted a proposal for a regional Ag-SAT food safety conference to USDA. The second group identified regional resource personnel and potential rapid response teams. A third group inventoried regional extension and research publications in food safety. A proposal to form a development committee for a regional microbiology food safety project from the fourth group was approved by the Southern Region Experiment Station Directors. Current and future networking opportunities in the SERA-IEG will be discussed.

RAPID METHODS NETWORKING

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Rapid Methods and Automation in Microbiology is a relatively new and dynamic field in applied microbiology. The focal points of the field revolve around the series of International Symposium started in 1973. The seventh one will be held in London in 1993. The Kansas State University International Workshop on Rapid Methods and Automation is held every July. The 13th one was held in July, 1993. The cumulative directory has about 800 past participants to this lively workshop. This truly is a great networking system. Another contact point is the *Journal of Rapid Methods and Automation in Microbiology*. This journal accepts papers in all areas related to methodologies in applied microbiology.

The Food Consortium which involves the University of Arkansas, Iowa State and Kansas State and serves as a model of excellent cooperation among scientists in these institutions. The Consortium serves as a good focal point for networking and research cooperating among about 50 scientists. Food Scientists of all disciplines must work together for the common good to ensure food safety for all.

FOOD SAFETY NETWORKS IN CANADA

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In recent years, increasing consumer and Government interest in food safety issues has necessitated a re-thinking of the way in which research on food safety is co-ordinated and conducted. To make significant progress in food safety, it is clear that we must target broad problems across numerous commodity groups and departmental jurisdictions. Research will need to be conducted by large multi-disciplinary teams who have shed their allegiances and historical biases to various professional groups. In a world of constricting resources we cannot afford the numerous "me-to" projects, that have followed each new research swing in food safety. We will need to encourage the sharing of resources, expertise and equipment and reward those individuals and agencies that promote and take part in joint research ventures. This presentation will relate our experiences with the Agri-Food Safety Research Network of Agriculture Canada and also the Guelph Group for Research in Food Borne Pathogens. Networks offer a cost effective, low overhead way of facilitating collaboration of multi-disciplinary research teams, that will be essential for productive solutions to food safety issues.

FOODBORNE SAFETY APPLICATIONS OF THE PUBLIC HEALTH INFORMATION SYSTEM

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Historically the reporting of foodborne diseases has been characterized by a long interval between the disease event and the availability for analysis of the data describing the event. Implement of the Public Health Information System (PHLIS) by the Centers for Disease Control in over 45 public health departments in the U.S. has dramatically increased the ability to respond to food safety hazards. PHLIS is a PC based electronic reporting system for entering, editing, and analyzing data locally and transmitting data electronically to other sites. For foodborne disease, PHLIS is currently collecting information on *Salmonella*, *Shigella*, *Campylobacter*, *E. coli* O157:H7 infections and data to characterize the risk of seafood-associated disease. An additional module is being developed for reporting foodborne disease outbreaks. PHLIS has provided a mechanism to rapidly respond to inquiries about on-going outbreaks, including the potential to identify foodborne disease outbreaks. PHLIS is available to organizations or countries wishing to implement the system for their own data needs.

LATE BREAKING REPORTS: HAZARDS OF PROTOZOA IN FOOD AND WATER — THE CASE OF *CRYPTOSPORIDIUM*

ENTERIC WATERBORNE PROTOZOA: HAZARD AND EXPOSURE ASSESSMENT

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Risk is defined by an exposure to a particular hazard and the effects that hazard may have on the target population. Hazard assessment is also the first step in a quantitative risk assessment approach. For microbial agents, which are transmitted through contaminated water, this includes defining the occurrence of the pathogen in the population and in the water, as well as examining the data which relates the two.

Cryptosporidium and *Giardia* are enteric obligate protozoa and are now recognized as an important cause of waterborne disease. Giardia is the most frequently identified parasite associated with diarrhea in the United States and may be found in 5 to 12% of the population. It is estimated that 60% of the cases are water transmitted. This protozoan has also been responsible for 27% of the waterborne outbreaks in 1989 and 1990 with over 100 outbreaks reported since the first outbreak was documented in 1966. *Cryptosporidium* first emerged as a human pathogen in the 1980s and is now thought to be one of the third most common enteropathogens causing diarrheal illness. As there is no effective therapy for cryptosporidiosis, a preventative approach is required. The duration and the severity of the disease are significant, and there is emerging evidence that *Cryptosporidium* is also associated with hepatobilary and respiratory disease. There have been three waterborne outbreaks of cryptosporidiosis in the United States associated with well, spring and surface waters. The most recent two, prior to Milwaukee (1987 and 1992) caused widespread illness throughout the communities (55,000 and 5,000 illnesses, respectively). *Cryptosporidium* oocysts and *Giardia* cysts are commonly detected in surface supplies in the United States and have been detected in 27% and 17% of the treated drinking water samples, respectively. Human and animal infectious dose studies have demonstrated that low levels of cysts and oocysts are capable of causing infection. Low levels of these protozoa in treated drinking water are of a public health concern and models predict risks of 10^{-3} to 10^{-5}. An assessment of the sources, transport and survival of cysts and oocysts is needed to further characterize the hazard. This type of data will provide valuable information for development of appropriate risk management strategies.

FOODBORNE AND WATERBORNE PROTOZOA: PUBLIC HEALTH IMPLICATIONS

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Protozoa, particularly *Giardia lamblia* and *Cryptosporidium parvum*, are increasingly recognized as important causes of waterborne and foodborne disease in the United States. *Giardia* has been the most frequently identified etiologic agent in waterborne disease outbreaks for more than a decade, and several large waterborne outbreaks of cryptosporidiosis have occurred in recent years. Some of these outbreaks have been associated with municipal water supplies that met existing state or federal water treatment standards. Outbreaks of giardiasis and cryptosporidiosis have also resulted from recreational exposure to contaminated water in swimming pools. The most commonly recognized protozoal foodborne diseases include giardiasis and toxoplasmosis; two recent foodborne *Giardia* outbreaks occurred in commercial food establishments. Prevention and control of waterborne and foodborne protozoal disease raises several challenging public health issues. These include the need for revision of existing municipal water treatment standards and regulations and development of rational policies regarding testing and treating foodhandlers for protozoal infections.

CRYPTOSPORIDIUM: THE MILWAUKEE OUTBREAK

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Waterborne outbreaks of illness caused by Cryptosporidium have infrequently been associated with filtered surface water supplies that meet current federal or state water quality standards. Investigation of a massive outbreak of Cryptosporidium infections occurring in March-April, 1993 in a large city with two water treatment plants (north and south) has allowed us to examine these standards. The outbreak investigation has been extensive and has included examination of water quality indicators prior to the outbreak, hospital laboratory data, diarrheal rates among nursing home residents from geographical distinct sites, case follow-up studies and case control studies utilizing interviews of individuals with Cryptosporidium identified in stools and individuals receiving care at Milwaukee County emergency rooms for gastrointestinal complaints, surveys of individuals from the Milwaukee metropolitan area selected through random digit dialing techniques, and environmentally related studies to evaluate land use impacts. While treated (effluent) water leaving the plants met all regulatory agency quality standards, effluent turbidity at the south plant markedly increased in temporal association with the outbreak onset. Early in the outbreak, Cryptosporidium was identified in 71 (27%) of 266 stool specimens examined. Studies for bacterial, viral, and other parasites failed to implicate other pathogens. Case patients identified with Cryptosporidium infection typically experienced watery diarrhea and abdominal cramping. Nursing home residents receiving water from the south plant of the water district were significantly more likely to have experienced watery diarrhea. Results of the random digit dialing surveys indicate that greater than 39% of the population at risk in the water catchment area and 26% of persons in the five county metropolitan area may have been affected. This massive waterborne outbreak of cryptosporidiosis was associated with a break in the coagulation and filtration capacity of a public water supply that continued to meet regulatory standards.

WATER REUSE IN ANIMAL PROCESSING PLANTS SYMPOSIUM

WATER USE AND REUSE IN ANIMAL PROCESSING PLANTS

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The food industry is one of the largest and most important contributors to the U.S. economy. Increasing the ability of the U.S. to compete in the global economy is of national concern. There is also an increasing awareness of the need to responsibly manage and protect our environment: food processing plants are facing escalating costs and some may be threatened with closure as water sources, wastewater treatment, and solid waste facilities become inadequate. Because facing environmental challenges is crucial to food industry economic strength and employment, environmentally clean processing technologies are needed to combat pollution and conserve our vital natural resources without any reduction in productivity.

Many animal processing plants use in excess of 1,000,000 gallons of water per day. Water is used in a variety of processes including washing, conveying, scalding, cooling, cooking, sanitation, etc. Although, at present, water reuse is allowed only under prescribed conditions, many opportunities still exist in which reuse could conserve water. Specific concerns involving water reuse include water supply and quality, which are critical to food product quality and consumer safety.

Food scientists need to address environmental issues to strengthen the ability of food industries in the United States to protect the environment, assure a safe and nutritious food supply, and compete in the world economy.

WATER REUSE SYSTEMS: AN FSIS PERSPECTIVE

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Present FSIS policy requires the use of potable water on edible product and equipment that contacts edible product. Within this policy, nonpotable water and process water may be reused in specific instances. FSIS, EPA and FDA have discussed treating plant process water to return it to a quality that would allow extensive reuse in place of potable water. Treatment systems are being evaluated. One pilot test to treat and reuse large volumes of plant process water is being conducted. FSIS plans to announce formally its intention to allow the reuse of treated process water.

EPA'S MICROBIOLOGY REGULATIONS FOR DRINKING WATER

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The Office of Ground Water and Drinking Water, U.S. Environmental Protection Agency (EPA), is responsible for setting national requirements for controlling pathogenic microorganisms and toxic chemicals in drinking water. To control waterborne disease caused by microorganisms, EPA published two rules in 1989 — the Total Coliform Rule and the Surface Water Treatment Requirements — and is developing another rule, the Ground Water Disinfection Rule. In addition, EPA is developing a rule that will address the issue of toxic chemical byproducts that form when disinfectants used for microbial control in drinking water react with various organic chemicals in the source water. The challenge of this latter rule will be to set limits on the concentrations of toxic disinfection byproducts and perhaps the disinfectants themselves without undermining the control of pathogenic microorganisms.

DRINKING WATER ASSOCIATED WITH WATERBORNE DISEASE: HEMORRHAGIC COLITIS

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The enteric pathogen *Escherichia coli O157:H7* has emerged as an important causative agent of diarrheal disease. A recent disease outbreak associated with waterborne transmission of this organism resulted in four deaths, 32 hospitalizations and 243 documented cases of diarrhea. The pathogenic agent was isolated from patients' feces and illness was restricted to individuals using the public water supply. Deficiencies in the water distribution system were implicated as the source of the contamination. The outbreak subsided after chlorination was instituted in the system. Survival characteristics of this pathogen in water and it's antigenic relatedness to other species of the genus *Escherichia* will be discussed.

MECHANICAL DISINFECTION OF REUSE WATER IN POULTRY PLANTS

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Microfiltration may be used to disinfect poultry processing waters for reuse. Ceramic microfilters were used to treat carcass-chiller waters, scalder effluents, and process product chiller brines. Microfilters with pore sizes of 0.45 and 0.2 micrometers were both effective in reducing the microbial load of the treated effluents. Aerobic plate counts, using plate count agar (PCA), were reduced from 10^7 to <10 per ml. for scalder effluents, and from 10^5 to <10 for carcass chiller effluents. Similarly counts were reduced for chiller brines from about 10^5 to <10, using Staphylococcus 110 medium. While microfilters of the pore sizes tested essentially eliminated bacteria, which are about 0.5 to 10 microns in size, they would not be expected to eliminate viruses, which are about 0.05 microns or less in size.

CHLORINATION OF POULTRY CHILLER WATER — A MODEL OF ANIMAL PRODUCTS PROCESSING WATER

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Chlorine is presently the sole disinfectant allowed by the United States Department of Agriculture to be used in water that comes in direct contact with meats and poultry. During chlorination the dissolved matter in processing water not only reduces the bactericidal efficacy of chlorine but also reacts with chlorine to form oxidation and chloro-derivatives. Although most derivatives are yet to be characterized, their likeliness to increase during processing has been a primary concern of water reuse. Poultry chiller water was chosen as a model for the study of chlorination effects on processing water with and without reuse. Findings on the mutagonicity and composition of chlorinated water, chlorine demand and the efficacy of chlorine will be discussed.

FILTRATION AND RECONDITIONING OF PROCESS WATER FOR REUSE

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The objective of this presentation is to present an overview of past and present research findings on the reconditioning of food process waters. Many of the examples that will be cited in this presentation have involved the treatment of spent poultry process waters. Due to the similarities between poultry and other food process water effluents, many of the research findings involving poultry process waters are directly applicable to other spent food process effluents. My presentation will review several studies including one conducted by Carawan et al., (1974) that examined the reuse potential of combining the pretreated effluents from the final bird washer and carcass chiller for use in the gizzard splitter; a study by Rogers (1978) that evaluated several combinations of treatments (e.g. cyclonic desludgers, vibrating screens, flotation cells, filtration through diatomaceous earth (DE), activated carbon, etc.) for reconditioning poultry chiller effluents; a series of studies by Lillard (1978a,b,c) that explored the efficacy of DE filtration for recycling broiler chiller water; more recent studies by Sheldon and coworkers (1988, 1989 a,b,c) that evaluated the economic impact and quality characteristics of overflow chiller water filtered through DE-coated pressure leaf filters; and filtration studies by Hart et al., (1988) that evaluated the effectiveness of cereaflow ceramic microfiltration bundles for clarifying poultry scalder and chiller effluents and frankfurter chiller brines.

Based on the USDA criteria established for recycling poultry chiller water for continued use on product, several of the treatment methods identified in this review satisfy current regulatory requirements for recycling. By far, the use of DE filtration appears to predominate the literature as one of the most effective and economical treatments for reconditioning food process water effluents. A number of potential benefits exist for food processors from initiating water conservation and recycling programs. They include: 1) improving the competitive position of the processor, especially those located in drought-stricken areas; 2) lowering processing costs through reduction in water use, wastewater surcharges, and energy costs; 3) reducing the environmental pollution potential; and 4) improving the public image of food processors.

MICROBIAL SAFETY OF USE OF RECONDITIONED PLANT WATER

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Throughout the United States there is an increased awareness of water as a limited resource. Animal processing plants use water throughout the entire operation and are required to use potable water on edible product and equipment that contacts edible products. Little information of the microbiological safety of the meat products was available for developing guidelines for permitting using reconditioned water in the plants. The Microbial Food Safety Research Unit of the ARS Eastern Regional Research Center has been working in cooperation with the USDA Food Safety and Inspection Service and the meat industry to assess the impact of using reconditioned water on the microbiological safety and quality of meat products. Results from a pilot study of pork carcasses indicated that the use of adequately reconditioned water does not sacrifice the bacteriological safety and quality of the carcass. Further studies are underway to determine the feasibility of expanding the use of reconditioned water in other meat processing operations.

INDUSTRY'S POINT OF VIEW FOR USE OF RECONDITIONED PLANT WATER

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Water is a critical natural resource whose conservation is increasingly important to the nation. Packing and processing operations require large volumes of water. For example, it is estimated that cattle slaughter uses approximately 500 gallons per head. Anything that can be done to conserve water, particularly through reuse, will result in not only conservation, but lower costs to the industry.

There have been several instances of water reuse programs at packing and processing plants which have been implemented with the approval of USDA/FSIS. FSIS has no formal procedure for evaluating such programs and those that have been approved were done on an ad hoc basis. We believe that if a formal procedure for developing a water reuse program was in effect, more companies would take the opportunity to use it. A letter was sent to FSIS last fall encouraging them to establish a protocol as soon as possible and, in the interim, make decisions about the pending petitions. Subsequent to the letter a meeting was held with FSIS to discuss the status of several pending water reuse petitions and the need for "guidelines" to be developed. The Agency agreed to take immediate action on the petitions. In addition, FSIS will work closely with AMI to implement formal guidelines on water reuse.

The Environmental Committee of AMI has established a subgroup whose goal is to work with USDA in order to provide input and encourage the development of guidelines for a water reuse program for the packing industry. Water reuse is an excellent example of combining pollution prevention, water conservation, and economic benefits to make good sense. The industry will work with all involved to ensure that human health is protected.

SCIENTIFIC POSTER SESSION

EVALUATION OF DIFFERENT MEDIA FOR RECOVERY OF THERMALLY-INJURED *ESCHERICHIA COLI* O157:H7

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Efficacies of plating media for recovering heated *Escherichia coli* O157:H7 were determined and compared. Suspensions of cells (3 isolates, 4 rep./isolate) were heated at 50, 55 or 60°C, then inoculated onto eight media; PCA-PA (plate count agar with 1% pyruvic acid [PA]), MSA (MacConkey sorbitol agar), MSA-Mg (MSA with 0.025% $MgSO_4$), MSA-PA (MSA with 1% PA), MSA-MUG (MSA with 0.005% 4-methylumbelliferyl-B-D-glucuronide (MUG), PRSA-MUG (phenol red sorbitol agar (PRSA) with 0.005% MUG), PRSA-PA (PRSA with 1% PA), and TSA-PA (tryptic soy agar with 1% PA), to compare populations of recovered cells. Recovery was consistently higher ($p<.05$) with PRSA-MUG and PRSA-PA. At 50, 55 and 60°C, mean numbers (log_{10} CFU/ml) of recovered cells on PRSA-MUG were 4.42, 4.62, and 3.32, respectively as compared to 2.78, 2.08, and 1.63, respectively on MSA. PCA-PA and TSA-PA were less effective than PRSA media, but better than MSA media. Thus, PRSA with MUG or PA is an effective medium for recovering heated cells and differentiating *E. coli* O157:H7; whereas, MSA fails to detect sublethally-injured cells. Furthermore, addition of Mg, PA or MUG to MSA further compromises this medium.

FATE OF ENTEROHEMORRHAGIC *ESCHERICHIA COLI* O157:H7 IN UNPASTEURIZED APPLE CIDER WITH AND WITHOUT PRESERVATIVES

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A strain of enterohemorrhagic *Escherichia coli* O157:H7 isolated from a patient in an apple cider-related outbreak was used to evaluate the fate of *E. coli* O157:H7 in six different lots of unpasteurized apple cider. In addition, the efficiency of two preservatives, 0.1% sodium benzoate or 0.1% potassium sorbate, used separately and in combination was evaluated for bactericidal effects on *E. coli* O157:H7. Studies were done at 8° or 25°C in cider with pH 3.6 to 4.0. Results revealed that *E. coli* O157:H7 populations increased slightly (ca. 1 log_{10} CFU/ml) in some but not all lots. Survival of the organism, (initial population of 10^5 CFU/ml) ranged from 10-31 days at 8°C and between 2-3 days at 25°C. Potassium sorbate had minimal effect on *E. coli* O157:H7, with survivors detected from 15-20 days or 1-3 days at 8° or 25°C, respectively. In contrast, survivors in cider containing sodium benzoate were detected for only 2-10 days or less than 1-2 days at 8° or 25°C,

respectively. Greatest rates of inactivation occurred in the presence of both sodium benzoate and potassium sorbate. The use of 0.1% sodium benzoate, an approved preservative that some cider processors occasionally use, will substantially increase the safety of apple cider relative to *E. coli* O157:H7, in addition to suppressing the growth of yeasts and molds.

STORAGE TEMPERATURE AND HEAT RESISTANCE OF *ESCHERICHIA COLI* O157:H7 IN GROUND BEEF PATTIES

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The presence of pathogenic bacteria such as *E. coli* O157:H7 and *Salmonella* in ground been has prompted requirements for extensive heat treatments of ground beef patties to ensure safe consumption of hamburgers in foodservice establishments. The temperature of storage and the temperature of a raw food immediately before cooking can significantly affect the heat sensitivity of certain pathogenic bacteria. This study has sought to determine if the storage of *E. coli* O157:H7 at different temperatures and pre-incubation after cold storage at elevated temperatures affects the subsequent heat sensitivity of the organism in commercially-used heat treatments for ground beef patties. Cultures were stored at -20°C, 4°C, and 15°C in nutrient broth. Thermal resistance at 55°C was determined for stored samples, as well as stored samples pre-incubated at 22°C and 30°C for up to 4 hours prior to heating. In addition, the influence of these storage conditions on the survival of *E. coli* O157:H7 during the cooking of ground beef patties of various fat levels was investigated.

GROWTH OF *ESCHERICHIA COLI* O157:H7 IN GROUND, ROASTED BEEF AS AFFECTED BY PH, ACIDULANT AND TEMPERATURE

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The fate of *Escherichia coli* O157:H7 in ground, roasted beef as influenced by pH, acidulant, temperature and time was studied. Populations did not change when beef salads (pH 5.40 to 6.07) containing up to 40% mayonnaise were incubated at 5°C for up to 72 h. At 21 and 30°C, significant ($P \leq 0.05$) increases in populations occurred in salads containing 16 to 32% mayonnaise (pH 5.94 to 5.55) between 10 and 24 h of incubation. Death was more rapid at 5°C as the pH of beef slurries acidified with acetic, citric and lactic acids was decreased from 5.98 to 4.70. *E. coli* O157:H7 grew in control slurries (pH 5.98) and in slurries containing citric and lactic acids (pH 5.00 and 5.40) incubated at 21°C for 24 h. At 30°C, populations decreased in slurries acidified to pH 4.70 and 5.00 with acetic acid but increased in slurries acidified to pH 4.70 with citric and lactic acids. The order of effectiveness of acidulants in inhibiting growth and inactivating *E. coli* O157:H7 in beef slurry (pH 5.00) heated at 54°C was acetic>lactic≥citric acid. Caution should be taken when preparing, distributing and marketing beef salads so as to prevent cross contamination from uncooked foods, since *E. coli* O157:H7 can grow at pH values characteristic of beef salads.

COMPETITIVE GROWTH IN BIOFILM OF *L. MONOCYTOGENES* WITH CULTURES ISOLATED FROM A MEAT PLANT ENVIRONMENT

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Growth of *L. monocytogenes* Scott A with competitive cultures obtained from a meat processing plant environment was studied in biofilms using two low nutrient media (0.2 and 1% tryptic soy broth) and two temperatures (10 and 21°C). Stainless steel slides (SSS) were periodically transferred to fresh media to produce the biofilm. Growth of *L. monocytogenes* in biofilm was observed in most experiments. The existence of antimicrobial substances produced by *Streptococcus* significantly inhibited growth of *L. monocytogenes* in broth and its attachment to SSS, but not its growth in biofilm. Also, increased growth of *L. monocytogenes* in the presence of *Pseudomonas* was observed in biofilms. Formation of biofilms on SSS might prevent antimicrobial substances produced by competitive cultures from reaching imbedded cells resulting in lack of inhibition of growth of *L. monocytogenes*.

INTERACTIONS OF DIACETATE WITH NITRITE, LACTATE, AND PEDIOCIN ON VIABILITY OF *LISTERIA MONOCYTOGENES* IN TURKEY SLURRIES

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Sodium diacetate (0 to 0.5%), alone or in combination with sodium nitrite (final 30 ppm), sodium lactate (2.5% v/v), or pediocin (5,000 AU/ml), was added to turkey slurries (25%) containing *Listeria monocytogenes* (10^4 CFU/ml). Pathogen levels reached ca. 10^8 CFU/ml in slurries containing nitrite or lactate at 4 (14 d) and 25°C (24 h). In the presence of pediocin, listeriae reached ca. 10^7 CFU/ml at 25°C (24 h), but at 4°C, counts of the pathogen declined ca.1 log 10_{10} unit and did not reach elevated levels (10^8 CFU/ml) for 28 d. In contrast, concentrations of 0.3% and 0.5% diacetate were bacteriostatic at 4 and 25°C, respectively. No additional antilisterial effect was observed using nitrite in combination with diacetate, whereas lactate and diacetate (0.1%) were bacteriostatic and pediocin and diacetate [0.3% (4°C); 0.5% (25°C)] were listericidal. These results revealed that diacetate can be used to delay the growth of *L. monocytogenes* in turkey, and that an additional level of safety can be achieved using diacetate in combination with other preservatives, notably lactate and pediocin.

MICROBIAL INHIBITION OF *LISTERIA MONOCYTOGENES* BY OTHER BACTERIA IN A COMMERCIAL MILK AND A BUFFER BROTH SYSTEM

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When *Listeria monocytogenes* and *Lactobacillus bulgaricus* (ATCC 27558) were cultured together, no inhibition of *L. monocytogenes* was seen in either regular milk or in a buffered broth. However, upon co-culture of a Gram-positive coccus, isolated from the bulgarian style culture milk and *L. monocytogenes*, the growth of *L. monocytogenes* was significantly inhibited. The inhibition was observed to a lesser degree when the two organisms were co-cultured in tryptose phosphate broth (TPB), a pH buffered broth. Data suggest that *L. monocytogenes* was inhibited by a substance or substances produced by this Gram-positive coccus. Therefore, the potential of using this Gram-positive organism to control the growth of *L. monocytogenes* in cultured milk may be promising.

INTERACTION OF CITRIC ACID CONCENTRATION AND PH ON THE KINETICS OF *LISTERIA MONOCYTOGENES* INACTIVATION

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The interaction between pH and citric acid concentration on the inactivation of *Listeria monocytogenes* was determined using a three strain mixture. Citric acid/sodium citrate combinations were added to BHI broth to achieve concentrations of 0.1, 0.5, 1.0, and 2.0 M in conjunction with pH values 4, 5, 6, and 7. The media were dispersed in 20-ml portions in dilution bottles, inoculated to 10^8 cfu/ml, and incubated aerobically at 28°C. Survivor curves were generated using a linear model incorporating a lag term, and D-values and 4D-inactivation times calculated. The results were compared against control cultures with HCL. The rate of inactivation was dependent on both the pH and concentration of citric acid. Low levels of citric acid were protective particularly at pH 5 and 6. At higher concentrations, a distinct anion effect was observed as compared to the HCL controls. Comparison of the kinetic data with earlier results with lactic and acetic acids suggests that citric acid may be inactivating *L. monocytogenes* by a different mode of action, or involves multiple sites.

COMPARATIVE GROWTH RATES OF *LISTERIA MONOCYTOGENES* ON RAW AND COOKED MUSCLE TISSUES

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The growth rate of *Listeria monocytogenes* on raw and cooked beef, chicken, catfish and shrimp was compared. Uncooked samples were inoculated, overwrapped with PVDC and stored at 4°C. Other portions were fully cooked, inoculated, overwrapped with PVDC and stored at 4°C. Samples were analyzed for 11 days for the *L. monocytogenes* and total psychrotrophic populations using Modified Oxford *Listeria* Agar and Plate Count Agar, respectively. The pH of the samples was determined at each sampling time. Growth of *L. monocytogenes* occurred at a faster rate and reached a higher population on raw catfish and shrimp than on raw beef or chicken. On the

cooked samples, *L. monocytogenes* population was 1 log greater on catfish and 2 logs greater on shrimp than on beef and chicken. The total psychrotrophic population was 1 log greater on beef and chicken than on catfish and shrimp after 11 days regardless of whether the muscle was raw or cooked. Slight differences in the pH of raw and cooked muscle types were noted but do not appear to solely account for differences in *L. monocytogenes* growth rates on the muscles.

GROWTH OF *LISTERIA MONOCYTOGENES* AT FLUCTUATING TEMPERATURES

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The effect of temperature on *Listeria monocytogenes* growth in Brain Heart Infusion Broth (pH 6.0) was investigated at 2 NaCl levels (0.5% and 6.5%). Three fluctuating temperature regimes were used. (12°C-28°C-12°C; 19°C-28°C-19°C; 19°C-37°C-19°C, adjusted every 6 hours). Growth was also measured isothermically at 12°C, 19°C, 24°C, 28°C, 32°C, and 37°C. Generally, fluctuating temperatures resulted in longer lag phase durations than when cells were grown isothermically at the midpoint temperatures. Generation times were generally less than those obtained when cells were grown isothermically at the midpoint temperatures. The extent of the differential depended on starting temperature and NaCl level. Effective mathematical models for predicting pathogen growth in foods will have to account for these effects.

COMPARISON OF METHODS FOR ISOLATION OF *LISTERIA* FROM RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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Rainbow trout samples (30), purchased at retail markets, were surveyed for the presence of *Listeria*. Samples were directly plated and enriched following the USDA and FDA protocols. Plating was performed on PALCAM and Modified Oxford agar (MOX) after 24 and 48 h. A total of 13 samples (43%) tested positive for *Listeria* at one of the plating periods. Three positive samples (23%) were detected by direct plating on MOX agar; none were detected on PALCAM. One of the positive samples detected by direct plating tested negative after enrichment. A total of 12 positive samples (92%) were detected by the FDA procedure, while the USDA procedure detected 10 positive samples (77%). Neither procedure detected all of the positive samples at either time period. PALCAM was more selective and resulted in more isolated colonies after 24 h enrichment, but did not yield any colonies during direct plating. From these results, it was concluded that no one procedure is superior for isolation of *Listeria* from fresh trout, and the best isolation can be obtained by use of multiple enrichment and plating media.

ENHANCED RECOVERY AND ISOLATION OF *LISTERIA* USING A NOVEL CULTURE AND TRANSFER DEVICE

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A novel transfer-inoculation device for improved detection of *Listeria monocytogenes* in food and environmental samples was evaluated. Samples were analyzed by standard FDA and USDA procedures. The only modification to the standard cultural procedures was utilization of the transfer-inoculation device. A total of 224 samples in 6 trials on 6 foods were analyzed by FDA cultural, FDA with device, USDA cultural, and USDA with device for *L. monocytogenes*. Detection of *L. monocytogenes* with use of the device was improved by >31% over the standard FDA cultural method and by >10% using the USDA cultural method. The USDA method detection incidence was 71.9% greater than the FDA method. A second blind field test in a routine testing laboratory used samples potentially naturally-contaminated with *Listeria monocytogenes*. To date, a total of 56 food and environmental samples have been analyzed for *L. monocytogenes*. Detection of *L. monocytogenes* was improved by 20% over the standard USDA cultural method. The improvement in performance was statistically significant at $p \leq 0.0001$. There were no false-negative results for any analyses using the transfer-inoculation device.

COMPARISON OF OXYGEN SCAVENGERS FOR THEIR ABILITY TO ENHANCE RESUSCITATION OF HEAT-INJURED *LISTERIA MONOCYTOGENES*

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The recovery of heat-injured *Listeria monocytogenes* Scott A in Fraser broth supplemented with three concentrations each of sodium thioglycolate, sodium pyruvate, L-(+)-cysteine HCl, catalase and Oxyrase® was studied. After 3 h of incubation at 30°C, recovery was enhanced by all oxygen scavengers except sodium pyruvate; Oxyrase (0.005µ/ml) promoted the highest recovery (34.7%) compared to recovery in control broth (14.8%). The percentage recovery was increased as the incubation time was extended to 6 and 24 h. After 6 h of incubation, 52.9% and 55.8% of injured cells underwent resuscitation in broth containing 0.25% sodium pyruvate and 0.04% catalase, respectively, compared to 25.8% resuscitation in broth not supplemented with oxygen scavengers. Results indicate that all oxygen scavengers tested enhanced the recovery of injured *Listeria monocytogenes* in Fraser broth within 6 h of incubation. Nearly all injured cells were recovered with 24 h of incubation, regardless of type or concentration of oxygen scavenger in Fraser broth. The incorporation of one or more oxygen scavengers into Fraser broth for the purpose of promoting recovery of heat-injured *L. monocytogenes* cells holds promise as a method to enhance the sensitivity of procedures for detecting the pathogen in heat-processed foods.

ADVANCED GENOTYPIC TYPING OF *LISTERIA MONOCYTOGENES* USING CLAMPED HOMOGENEOUS ELECTRIC FIELDS (CHEF) ELECTROPHORESIS

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Clamped homogeneous electric fields (CHEF) electrophoresis was optimized for *Listeria monocytogenes* and a data management program was used for profile comparisons. Various food and clinical isolates, including mother-baby pairs (MB), multiple isolates from the same source (MI), and isolates from both food-related outbreaks and sporadic cases were digested with rare-cutting endonucleases. Of 150 strains analyzed, *Asc*I, *Apa*I, and *Sma*I established 58, 66, and 58 CHEF profiles, respectively. With few exceptions, strains of different serovar and strains within each serovar displayed different CHEF profiles. However, serovar 1/2a, 1/2c, and 3a strains exhibited similarities in their genomic fingerprints, and similarities were also evident among serovar 1/2b, 3b, and 4b strains. CHEF analyses revealed 9 of 13 MB and 12 of 17 MI displayed identical fingerprints, whereas non-identical MB and MI displayed only minor (1 or 2 bands) differences in profiles. These data substantiate that CHEF analyses is a reproducible and highly discriminatory method for molecular tracking of *L. monocytogenes*.

DETERMINING DIFFERENCES IN MICROBIAL GROWTH RATES USING LINEAR REGRESSION

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The aim of this study was to present a simple, statistically correct, method for comparing growth rates. Two different data sets were used to test the statistical method. The first set contained data on the growth of *Listeria monocytogenes* Scott A PFEI and *Listeria innocua* PFEI. At most of the temperatures (2, 3, 5, 15, 20, 35, 37 and 40°C) the growth rates of *L. innocua* and *L. monocytogenes* were best described by a model which assumed that growth rates and lag times were significantly ($p<0.05$) different. At some temperatures (8, 10, 25, 38, 42 and 44°C) a model which assumes that growth rates are not significantly different best described the data. A second set contained data on growth of *L. innocua* at 37°C with conventional heating at the same temperature in a specially designed microwave oven. It was found that growth rates were not significantly different under each heating condition. This study successfully demonstrated the utility and suitability of a simple statistical method to compare and discriminate between bacterial growth rates.

ACID ENHANCEMENT OF *CLOSTRIDIUM PERFRINGENS* SPORULATION

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Ingestion of *C. perfringens* followed by its sporulation and enterotoxin release may result in a mild diarrheal disease. In vitro, sporulation is

problematic. Sporulation media are complex and do not support the sporulation of all enterotoxin strains. In vivo, the organism sporulates in the intestines after travelling through the stomach. In this study the effect of short term acidic conditions on sporulation was examined. *C. perfringens* (ATCC 12915) cultures, in thiogly-collate medium, were divided into samples. The pH of the samples was adjusted with 1 N HCl. At regular intervals the samples were neutralized and transferred to Duncan-Strong sporulation broth. Following a 20 hr incubation, the presence of heat resistant spores was determined. Extreme acidic conditions (pH 2) for 2 hr inhibited sporulation. Shorter treatments (30 - 60 min) resulted in enhanced sporulation when compared to controls. In milder conditions (pH 3.5-4) the bacteria also exhibited enhanced sporulation. An implication of the data is that the acidity of the stomach and the duration of contaminated food in the stomach may play a role in C. perfringens food poisoning.

THERMAL RESISTANCE OF SPORES OF NON-PROTEOLYTIC TYPE B AND TYPE E *CLOSTRIDIUM BOTULINUM*

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A significant concern with cook/chill foods is the survival and growth of non-proteolytic *C. botulinum*, which can grow at temperatures as low as 3.3°C. Therefore, the heat resistance of non-proteolytic type B and type E *C. botulinum* spores was determined at 70, 75, and 80°C. Spore suspensions were prepared in trypticase-peptone-glucose-yeast extract broth, washed, and resuspended in phosphate buffer. Heated spores were enumerated on Reinforced Clostridium Medium (RCM) and Tryptic Soy Agar with and without lysozyme (10 µg/ml). Decimal reduction times (D) were determined by linear regression analysis of data. Spores of non-proteolytic type B strain had greater thermal resistance than type E strain. Apparent or measured heat resistance was maximum when the recovery medium was RCM with lysozyme. D-values for non-proteolytic type B were 52.8 min at 70°C, 14.1 min at 75°C and 9.76 min at 80°C (Z= 13.6°) and those for type E were 8.80 min at 70°C, 3.64 min at 75°C and 2.55 min at 80°C, (Z=18.6°C). These data will assist food processors to design thermal processes that ensure safety against non-proteolytic *C. botulinum* in cook/chill foods.

EFFECT OF SODIUM LACTATE ON TOXIGENESIS OF *CLOSTRIDIUM BOTULINUM* IN 'SOUS-VIDE' PRODUCTS

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The effect of sodium lactate (L) on toxigenesis of *Clostridium botulinum* as affected by storage temperature (T) in three 'sous-vide' products (beef, chicken breast, and salmon) was evaluated. The beef and salmon were homogenized with 0, 2.4, and 4.8% L (w/w), whereas the chicken was homogenized with the same levels of L (wet basis). Homogenates were inoculated with 10^4 spores of a pool of nonproteolytic types B and E of *C. botulinum* (strains B2, B17, B197, B706, E211, E250, E KA-2, and E Beluga), and incubated at 4, 8, 12, and 30°C; or inoculated with 10^4 spores of a pool of proteolytic types A, B, and F (A62, A69, AFT48, ARS3, B133, B OKRA, FFT42, and FPC), and incubated at 16, and 30°C, under vacuum for up to 90 days. Sodium lactate (2.4%, and 4.8%) delayed the time of toxicity by ≥5 and ≥82 days, at 30 and 8°C, respectively. The antimicrobial effect of L was enhanced extensively by the lowering of T. This study demonstrated that increasing L concentrations and lowering of T had significant beneficial effect on delaying toxigenesis of *C. botulinum* in the 'sous-vide' products.

RELATIONSHIP OF *VIBRIO* SPP. IN SOFT CLAMS AND WATER WITH *CLOSTRIDIUM PERFRINGENS* AND FECAL INDICATORS

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Analyses of Venezuelan soft clams, coastal seawater, and fresh water were performed to determine the presence of *Vibrio*-like spp., *V. parahaemolyticus*, *V. vulnificus*, and *V. cholera*. Additionally, total microbial levels, and levels of *C. perfringens*, total coliforms and fecal coliforms in these samples were evaluated in an effort to determine a relationship between incidence of these species, coliforms and *Vibrio* spp. *Vibrio* counts in soft clams and all waters range from 3.0×10^1 to 4.1×10^6 *Vibrio*-like cfu/g or ml. In all samples, total coliforms and fecal coliforms ranged from 0 to 2.4×10^5 /g or ml; *C. perfringens* counts range from 1.1×10^1 to 4.6×10^6/g or ml. In river water, the level of fecal coliforms ranged from 2.1×10^1 to 2.4×10^4/ml. A statistically significant relationship was found between *Vibrio*-like spp. and *C. perfringens* counts in soft clams and seawater. No relationship was found between fecal coliform counts and *Vibrio*-like counts in the same samples or between *C. perfringens* and *Vibrio* spp. in river water.

CONTROL OF THERMOPHILIC SPORE ACTIVITY WITH PRESSURIZED CARBON DIOXIDE AND EGG WHITE LYSOZYME

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The shelf life of low-acid, thermally processed military rations, such as stews and mixed vegetables, can be limited by the activity of gram-positive, heat-resistant sporeformers, such as *Bacillus stearothermophilus*. For that reason, an investigation was initiated to evaluate the feasibility of using a combination treatment of carbon dioxide (CO_2) and egg white lysozyme to inactivate foodborne thermophilic spores. Results show that when heat-resistant spores (10^2 and 10^4/mL) of *B. stearothermophilus* were cultured in AAMS broth (antibiotic assay medium + 0.1% soluble starch, pH 6.8;) treated with CO_2 (300 psi) and egg white lysozyme (200 micrograms of lysozyme/mL of broth) for 4 h at 55°C, no survivors were recovered when plated on AAMS agar. However, when CO_2 treatment was not prolonged, there was no apparent effect on the viability of 10^4 spores/mL, but the viability of 10^2 spores/mL was destroyed. It would appear from the foregoing that a combination treatment such as described would have some application in controlling the spoilage activity of thermophilic spores in thermally processed ration items.

CHEMICAL CHANGES OF PRE-PACKAGED SHEEPHEAD DURING FROZEN STORAGE

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Film overwrapped or vacuum-skin packaged dressed Sheephead (*Archosargus probatocephalus*) was stored at -28°C for one year. Vacuum-skin packaging treatments included "film-to-tray" with either oxygen barrier or permeable films, or "film-to-film" with oxygen barrier films. Fish quality changes were determined by TBA number, free fatty acid (FFA) level, pH, and ammonia production at 0, 3, 6, 9, and 12 months of storage. Mean proximate composition of the fish was 16.70% protein, 2.80% fat, 2.02% ash, and 78.24% moisture content. Fish muscle TBA and FFA levels increased with storage time regardless of package type. TBA numbers of the vacuum-skin packaged fish were significantly lower than those determined for overwrapped fishes at the 12th month. No significant differences in FFA levels, pH and ammonia production were found during storage.

EFFECTS OF TRISODIUM PHOSPHATE AND LACTIC ACID ON MICROBIOLOGICAL AND PHYSICAL QUALITY OF PACKAGED RAINBOW TROUT

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Rainbow trout (*Oncorhynchus mykiss*) was headed, gutted and dipped in water (control, pH 7.0; 1 min), 2% lactic acid (pH 2.3; 1 min), 2% lactic acid with 2% sodium chloride (pH 2.0; 1 min), or 10% trisodium phosphate (pH 12.5; 10 min). Treated samples were vacuum-skin packaged and stored at 4°C. Samples were evaluated after 0, 4, 8, 12, 16, and 20 days for total aerobic and anaerobic bacterial counts, surface pH, Torrymeter reading, and Hunter color values (L, "a", "b"). Fish dipped in 2% lactic acid had approximately 0.5 to 1.0 log lower aerobic and anaerobic populations at all sampling times followed by the combination of 2% lactic acid and 2% sodium chloride treatment, and the trisodium phosphate treatment. The control treatment had the highest microbial counts. Trisodium phosphate-treated fish exhibited higher pH, was darker, and had less redness and less yellowness values than other samples. No significant differences in the former attributes were found between lactic acid-treated and control samples throughout the entire storage period.

ANTIMICROBIAL CONTAINING EDIBLE FILMS AS AN INHIBITORY SYSTEM TO CONTROL MICROBIAL GROWTH ON MEAT PRODUCTS

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Microbial growth on raw and processed meat products is a major concern to the food industry. For this reason, a study was conducted to determine the effects of an edible coating containing antimicrobial agents on the growth of *Escherichia coli* O157:H7 and *Salmonella typhimurium*. Lactic,

acetic, propionic, acidified benzoic and acidified sorbic acids were chosen as possible antimicrobial agents. It was determined that a 0.30% solution of 20% potassium sorbate acidified to pH 4.7 with 10% lactic acid resulted in the greatest microbial growth reduction in a tryptic soy broth system. *S. typhimurium* had a four log reduction after only two hours of incubation at 37°C. By eight hours of incubation *S. typhimurium* was not recovered. *E. coli* O157:H7 had a two log reduction in growth after 3.5 hours of incubation. By 54.5 hours of incubation, no recovery of *S. typhimurium* was possible. An edible film consisting of water, starch, and glycerol was acidified with potassium sorbate for use in a shelf life study. Chicken breasts were overlayed with this film and stored at 7°C for 12 days. The addition of the film overlay did not significantly ($p>0.05$) decrease psychrotrophic counts on the chicken breasts. Shelf life studies did indicate that this edible coating can increase the shelf life appearance of chicken breasts by inhibiting a slimy appearance on the surface of the meat.

THE EFFECTIVENESS OF THE BACTERIOLYTIC ORGANISM, *BDELLOVIBRIO BACTERIOVORUS* 109J, AT REDUCING THE LEVEL OF GRAM-NEGATIVE FOODBORNE PATHOGENS

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Bdellovibrios are a group of aerobic, predatory bacteria which attack, penetrate and grow in many species of gram-negative bacteria, causing the lysis of the invaded host organism. The ability of *Bdellovibrio bacteriovorus* strain 109J to lyse 32 bacterial strains comprising six genera of foodborne pathogens and spoilage organisms was investigated. The reduction in the levels of the prey bacteria ranged from 0.1 to 7.7 log-values after 7 hours of incubation at 30°C. *Escherichia coli* strain 2239-69 (pathogenic serotype 026:H11) was lysed most effectively at temperatures between 30 and 37°C, however, lysis also occurred at 12 and 19°C when the incubation period was extended to 24 hours. *B. bacteriovorus* was effective in reducing the level of *E. coli* in the pH range of 5.6 to 8.6. Increasing the *Bdellovibrio:E. coli* ratio resulted in a more rapid reduction in the level of *E. coli*. This study demonstrated the potential usefulness of Bdellovibrios for the biological control of pathogenic and spoilage organisms in foods.

INHIBITION OF *SALMONELLA TYPHIMURIUM* BY THE LACTOPEROXIDASE SYSTEM IN A BROTH SYSTEM AND ON POULTRY

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The overall objective of this research was to evaluate the antibacterial activity of the lactoperoxidase (LP) system on survival and proliferation of *Salmonella typhimurium*. The LP system was found to have both bacteriostatic and bactericidal activities against *S. typhimurium* in tryptic soy broth (TSB). The bactericidal activity was clearly dependent on the initial inoculum level. At initial concentrations of 10^3 and 10^5 CFU/ml, a total bactericidal effect was evident after 5 and 15 h, respectively. For an initial inoculum level of 10^7 CFU/ml, a bactericidal effect was exhibited. However, after approximately a 4 log reduction over 16 h, growth proceeded as normal. The LP system enhanced thermal inactivation of *S. typhimurium* in TSB. The LP system reduced the D values by >80% at 50°C, 62.5% at 52°C, 71.4% at 55°C, 50% at 58°C, and 35.5% at 60°C. Chicken legs inoculated with *S. typhimurium* were immersed in a 25, 50, 55, or 60°C water bath containing the LP system for 5, 15, or 30 min. Reduction values varied according to time and temperature. The greatest reduction (80.6%) was seen at a treatment of 60°C for 15 min. Hunterlab color values for chicken thigh skin and TBA values for chicken thigh meat did not significantly differ ($p<.01$ and $p<.05$, respectively) between the control and treatment thighs.

EFFECT OF NACL OR WATER CONTENT ON THE SURVIVAL OF *SALMONELLA TYPHIMURIUM* ON IRRADIATED MEAT

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This study was initiated to investigate the effects of water content, water activity, or NaCl content on the survival of *Salmonella typhimurium* ATCC 14028 on irradiated mechanically deboned chicken meat or pork loin. The effects of NaCl were investigated by the addition of various amounts to mechanically deboned chicken meat or by the addition of various mixtures of NaCl solutions to freeze-dried ground pork loin. The effects of water contents on survival of *S. typhimurium* were investigated by rehydration of freeze-dried ground pork loin to various water contents. Inoculated samples were irradiated at 5°C *in vacuo* to various doses up to a maximum of 6.0 kGy. Water content, water activity, and NaCl significantly affected survival of *S. typhimurium*. The results indicated that the survival of food borne pathogens in irradiated meats with reduced water content, water activity, or increased NaCl levels may be greater than expected.

ATTACHMENT OF *SALMONELLA TYPHIMURIUM* AND *CAMPYLOBACTER JEJUNI* TO SKINS OF CHICKEN SCALDED AT VARIOUS TEMPERATURES

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Microtopography of chicken skin was manipulated by varying scalding temperature to find the least favorable skin surface for bacterial attachment. Chickens were processed using 52, 56, and 60°C scalding temperatures, and the changes in skin morphology were examined by light and transmission electron microscopy. Breast skins obtained after picking were inoculated with 10^8 CFU/ml of *S. typhimurium* or *C. jejuni*, and the attached cells were enumerated by using scanning electron microscopy. Skins scalded at 52 and 56°C retained most of epidermis after picking, although at the latter temperature, twice as much stratum corneum layers were lost which resulted in a smoother surface. Skins processed at 60°C lost most of the epidermal layers during scalding and the exposed dermal surfaces after picking Both *S. typhimurium* and *C. jejuni* were at least 1.0 to 1.4 log higher in 60°C-processed skin than in 52 and 56°C-processed ones. Above results suggest that these pathogens prefer dermis for attachment. Thus, the removal of whole epidermis should be avoided to reduce bacterial contamination during poultry processing.

EVALUATION OF A NITROCELLULOSE MEMBRANE LIFT METHOD FOR THE DETECTION OF *CAMPYLOBACTER* SPP. ATTACHED TO CHICKEN CARCASSES

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A nitrocellulose membrane-lift method to detect *Campylobacter* spp. attached to chicken skins was developed. In this method, pieces of nitrocellulose membranes of 0.45 µm, cut at 2.5 x 1.2 inches, were placed on skins of chicken carcasses and pressed by hand to ensure good contact between the membrane and the carcass surfaces. The membranes remained in contact with the chicken skins for 10 minutes and were removed. The membranes containing the lifted bacteria were directly placed on *Campylobacter* blood agar with the surface contact side up and incubated at 42°C microaerobically for 48 hours. Appearance of brownish, moist spreading colonies on membrane was considered a positive presumptive test for *Campylobacter* species. Immunostaining using peroxidase-conjugated *Campylobacter* spp.-specific antibodies was then performed directly on the membrane for the confirmation test. The NC lift method was effective and convenient as a nondestructive sampling method.

AN ELISA METHOD FOR THE DETECTION OF *CAMPYLOBACTER* IN RAW AND PROCESSED FOODS

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We have developed a rapid ELISA method for the identification of *Campylobacter* in processed foods following cultural enrichment. The assay is specific for *C. jejuni*, *C. coli*, *C. lari*, and *C. fetus* subsp. fetus. The ELISA reacted positively with 58/58 culturally confirmed isolates of *C. jejuni* and 7/7 culturally confirmed isolates of *C. coli* from retail meat and poultry. Many non-*Campylobacter* organisms were tested and found to be negative including: *Listeria*, *Salmonella*, *Streptococcus*, *Staphylococcus*, *Acinetobacter*, and *E. coli*. The assay is sensitive, detecting as few as 5×10^5 CFU/ml in pure culture. In frozen chicken nuggets spiked with 1 CFU of *C. jejuni* or *C. coli*, a positive result was determined within 48 h. In addition, sliced deli chicken spiked at 1 CFU/25 g gave similar results. This ELISA method for the detection of *Campylobacter* shows excellent sensitivity and specificity. It is simple to perform and can be used to test both raw and processed foods to ensure a *Campylobacter*-free product.

COMPARISON OF TECRA VIA KIT WITH OXOID AND CHO CELL ASSAY FOR THE DETECTION OF *BACILLUS CEREUS* DIARRHEAL ENTEROTOXIN

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A visual immunoassay kit (VIA; Tecra™, International BioProduct, Inc., Redmond, WA) and a reversed-passive latex agglutination kit (BCET-RPLA; Oxoid™, Unipath Co., Columbia, MD) were tested to assess their ability to detect *B. cereus* diarrheal (heat-labile) enterotoxin in cell-free culture filtrates from 12 isolates. Filtrates were also tested for biological activity against CHO cells (rounding and/or detachment). Positive (+) toxin responses by VIA correlated well with cytotonicity. High-titer (+) responses were observed using BCET-RPLA in 9/12 isolates, but 2 isolates that were (-) using BCET-RPLA produced (+) responses using VIA or cytotonicity. One isolate was strongly (+) with BCET-RPLA and CHO cell assays, but was weakly (+) with VIA. Boiling culture filtrates (10 min) eliminated (+) VIA and cytotonic responses for all isolates; however, boiled filtrates of 7/12 isolates were strongly (+) and 3/12 isolates were weakly (+) with the BCET-RPLA kit. Based on comparisons against the assay for biological activity, the VIA kit appears to have superior characteristics for the detection of *B. cereus* diarrheal enterotoxin.

EVALUATION OF RAPID TEST METHODS FOR DIRECT DETECTION OF *VIBRIO CHOLERAE* O1

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Cholera, caused by *Vibrio cholerae* O1, is spread through contaminated food and water. Rapid simple methods for testing environmental and food samples for *V. cholerae* O1 can be important in both surveillance and control programs. Three rapid test kits have been developed for direct detection of *V. cholerae* O1: CholeraScreen™, a co-agglutination test, Cholera SMART™, a colloidal gold based colorimetric test, and Cholera DFA, a direct fluorescent antibody test. The tests use a monoclonal antibody raised against the "A" component of the LPS of *V. cholerae* O1. Protocols for testing shrimp and water have been developed for these tests. The protocols involve filtration, enrichment in APW, then testing by either CholeraScreen™ or Cholera SMART™. Using these protocols, we were able to detect 10^2 CFUs/ml of *V. cholerae* O1 in a water sample within 6 hours and 1-10 CFUs/ml in water within 24 hours. Similar results have been obtained in testing food samples. Rapid test methods provide a valuable methodology for monitoring *V. cholerae* in environmental samples.

DETECTION OF COLIFORMS IN FOOD USING COLILERT-AN ASSESSMENT OF THE EFFECT OF DIFFERENT SUGARS FOUND IN VARIOUS FOODS

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Colilert is a Defined Substrate Technology™ which is used to simultaneously detect coliforms and *E. coli* in water and foods. Colilert contains ONPG and MUG as specific indicator nutrients to support the growth of the target organisms. Development of yellow color and blue fluorescence within 24 hours confirms the presence of coliforms and *E. coli* respectively. Previous studies have validated the use of Colilert primarily for water testing and also with certain foods. The possible use with other foods containing different sugars was investigated, since the preferential metabolism of those sugars might interfere with the Colilert reaction. D-glucose, D-sorbitol, lactose, mannitol, D-fructose, D-galactose, sucrose and corn syrup, at concentrations up to 10 g/L had no inhibitory effect on the production of yellow end-product (ONP) and fluorescence (4-MU) in Colilert inoculated with *E. coli* or *Enterobacter cloacae* at levels of 100, 50, 20, 10 and 1 cfu/ml. Therefore, the benefits of the Colilert test - rapid results, sensitivity and specificity offer many advantages for use in the food industry.

BIOLUMINESCENT METHOD FOR MEASURING TOTAL VIABLE COUNTS

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The bioluminescence from ATP catalyzed luciferin-luciferase reaction has been used as a highly sensitive monitor of total bacteria counts. Application of this method to monitoring bacteria counts has been limited by the instability of the reagents, the presence of inhibitory substances in many types of samples, and the cost of luminometry equipment. A method for immobilizing luciferin-luciferase and a small, hand-held luminometer have been developed which together overcome these obstacles. The sample is collected from a solid surface using a wipe pad or through filtration of a liquid sample eliminating free ATP and other substances which have been problematic in other forms of these assays. The high sensitivity and specificity of the system together with the portable nature of the instrument results in applications in many field monitoring conditions.

OCCURRENCE AND PRODUCTION OF ENTEROTOXIN PRODUCING STRAINS OF *STAPHYLOCOCCUS AUREUS* IN BAKERY PRODUCTS

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Bakery items were surveyed for the presence of *Staphylococcus aureus*. Items analyzed were Oatmeal/raisins cookies, apple muffins, cream puffs, and long johns. Coagulase-positive *S. aureus* were isolated from 21 (9.8%) of 214 bakery items surveyed. Enterotoxin was produced by 7 of these isolates. An enterotoxin producing *S. aureus* was found in one apple muffin and one long john and in 5 of the cream puffs. The pH and water activity ranges for the bakery items would support *S. aureus* growth except in the cookies. An enterotoxin A (SEA) producing strain of *S. aureus* was inoculated in or on the outside of the bakery items. The bakery items were held at 25°C for 48 h to simulate consumer handling. Total *S. aureus* was determined by plate count on Baird-Parker agar. *S. aureus* survived on all the bakery items. After 24 h, total cell number decreased on inoculated bakery items; however *S. aureus* grew well in the inoculated fillings of cream puffs and long johns increasing to 10^5 CFU/g. The SEA strain was inoculated at 10^4 CFU/g into apple muffin batter and held at 10°C for 120 h and 25°C for 48 h. *S. aureus* counts were determined every 24 h. Enterotoxin tests were conducted on samples with $\geq 10^6$ CFU/g. The 48 h, 25°C samples had counts of 10^8 CFU/g in the batter and tested positive for enterotoxin after baking. Counts did not increase in batter held at 10°C and baked muffins did not test positive for enterotoxin.

YEASTS ASSOCIATED WITH FRUIT JUICE CONCENTRATES

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Populations and identity of yeasts in frozen apple, cherry, grape, orange and pineapple juice concentrates were determined. Populations ranged from $\log_{10}$<1.00 to 5.41 cfu/ml of diluted (1:4) concentrate. Isolates (154) from 33 samples represented 21 species and 12 genera. The most frequently isolated species were *Saccharomyces cerevisiae* (24.7% of isolates), *Candida stellata* (22.1%) and *Zygosaccharomyces rouxii* (14.3%), followed by, in decreasing order of frequency, *Torulaspora delbrueckii*, *Rhodotorula mucilaginosa*, *Issatchenkia orientalis*, *Hanseniaspora occidentalis*, *Lodderomyces elongisporus*, *Kluyveromyces thermotolerans*, *Hanseniaspora guilliermondii*, *Candida glabrata* and *Pichia anomala*, each representing 3-8% of isolates. *Candida magnoliae*, *C. maltosa*, *C. parapsilosis*, *C. tropicalis*, *Clavispora lusitaniae*, *Cryptococcus humicolus*, *C. laurentii*, *Pichia membranaefaciens* and *Sporidiobolus salmonicolor* were represented by single isolates. Populations in various samples consisted of 24-100% *S. cerevisiae*, 52-100% *C. stellata* and 3-56% for *Z. rouxii*. This may be the first observation of this high frequency of *C. stellata* in juice concentrates.

USE OF AEROBIC PLATE COUNTS INCUBATED AT ELEVATED TEMPERATURES FOR DETECTING TEMPERATURE-ABUSED REFRIGERATED FOODS: EFFECTIVENESS UNDER TRANSISTORY ABUSE CONDITIONS

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Previous research has suggested that aerobic plate counts (APCs) incubated at elevated temperatures can be used to microbiologically differentiate temperature abused refrigerated foods. This approach was evaluated further by assessing its ability to detect transitory temperature abuse of raw and cooked shrimp. The shrimp were purchased from retail markets, and then stored under various regimes wherein the products were abused for one or more cycles at 12° or 19°C, and compared against samples stored at constant temperatures of 5° and 12° or 19°C. Using 42°C APCs, differentials between transitorily abused and adequately refrigerated samples were noted, but were

minimal for distinguishing marginally abused samples from those refrigerated for extended periods. The assay's effectiveness was enhanced by increasing the incubation temperature to 45°C. The results suggest that APCs incubated at appropriate elevated temperatures can be an effective microbiological indicator of temperature abuse in refrigerated foods.

ASSESSMENT OF PREVIOUS HEAT TREATMENT OF BEEF AND PORK PRODUCTS USING A DRY CHEMISTRY ENZYME SYSTEM

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A dry chemistry enzyme system "COBAS Ready" (Roche Diagnostics Systems) was used for assessing changes in enzyme (AST, CK and LDH) activity as influenced by previous heat treatment.

Laboratory and commercially prepared beef and pork products were assayed. Enzymes were extracted from the products with pH 7.0, 0.9% saline, filtered, and 20 µl of the filtrate used to determine AST, CK and LDH activity. Results are expressed as IU/L. High levels of CK, but very low levels of AST and LDH were detected in ground beef; however, high levels of AST, CK and LDH were found in uncured ground pork. High levels of AST were detected in imported canned hams, bologna and cooked salami products, with medium levels of CK being detected in those same products. No LDH activity was detected in commercially prepared cured products. Results indicate that the use of this enzyme system is product dependent.

FERMENTATION AND SENSORY CHARACTERISTICS OF KIMCHI CONTAINING KCL AS A PARTIAL REPLACEMENT FOR NACL

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There is an increasing desire by many consumers to lower their sodium intake. This has resulted in research activities devoted to partial or total replacement of NaCl with KCl in numerous food formulations. While reformulation without loss of sensory quality has been successful for several foods containing low concentrations of salt, the challenge of maintaining acceptable fermentation patterns and sensory quality development in fermented vegetables, for example, with normally elevated salt content is more difficult. A study was designed to determine the effects of substituting up to 50% of the NaCl in kimchi, a fermented Chinese cabbage (*Brassica pekinensis*) product containing scallions, garlic, ginger, hot red pepper powder and NaCl, with KCl. Brine water (15% salt) used to soak cabbage contained NaCl:KCl ratios of 1:0 (control), 5:1, 2:1 and 1:1 (wt:wt). Total acidity and pH of kimchi reached acceptable ranges of 0.4-0.6% (as lactic acid) and 4.4-4.7, respectively, after 13 days of incubation at 13±1°C. Kimchi made using brine water containing 5:1 and 2:1 (NaCl:KCl) salt ratios was characterized by faster growth of lactic acid bacteria and total aerobic microorganisms compared to the control formulation. Sensory qualities were acceptable.

CHARACTERIZATION OF ATTACHED, PSYCHROTROPIC BACTERIA ISOLATED FROM A WATER DISTRIBUTION SYSTEM

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Conventional methods for examining drinking water depend on the growth of indicator organisms as a measure of adequate disinfection practices. Unfortunately, these methods only account for planktonic microbes and not those organisms attached to the water distribution system. Because attached bacteria are generally less susceptible to disinfectants than planktonic cells, we characterized the attached bacteria that remained culturable in the presence of typical distribution system free-chlorine and chloramine residuals. Sterilized glass beads were initially suspended in untreated raw water for greater than 7 days to promote the adherence of indigenous bacteria. Beads were then placed in contact with continuously flowing streams of treated drinking water containing either free-chlorine or chloramines. At specified time intervals, beads were removed, rinsed with buffered water, placed in plate count- and R2A-broth, and incubated at 22°C for up to 72 hours. Bacteria were subcultured unto R2A plates and characterized using standard biochemical tests. Using numerical taxonomic techniques, bacteria were classified into groups based on similar biochemical properties.

DEGRADATION OF OCHRATOXIN A BY *ACINETOBACTER CALCOACETICUS*

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Microorganisms were screened for their ability to degrade ochratoxin A (OTA), and *Acinetobacter calcoaceticus* was found to degrade OTA. The degradation of OTA by *A. calcoaceticus* was studied in an ethanol-minimal salts medium with an initial OTA concentration of 10-50 µg/ml at 25°C and 30°C. *A. calcoaceticus* was able to degrade OTA in ethanol-minimal salts medium with initial concentrations of OTA of up to 40 µg/ml. At both temperatures, the growth of *A. calcoaceticus* in medium containing 50 µg OTA/ml was inhibited, and OTA degradation did not occur. The rate of OTA removed by *A. calcoaceticus* was concentration dependent; as the OTA concentration increased, the rate of OTA removed by *A. calcoaceticus* increased. OTA was degraded significantly ($p<0.05$) by *A. calcoaceticus* during and after the log phase of cell growth at both incubation temperatures. Hydrolysis of OTA by *A. calcoaceticus* yielded ochratoxin α, which is less toxic.

THE PHLS FOOD MICROBIOLOGY EXTERNAL QUALITY ASSESSMENT SCHEME

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Changing food legislation has prompted the UK Public Health Laboratory Service to launch an External Quality Assessment Scheme for food microbiology laboratories to assist them in overall quality assurance. The scheme involves the distribution of simulated food samples containing target organisms and associated background flora. The receiving laboratory selects its own methods for examination and reports on its findings within a defined period. Results are analyzed, scored and individual and overall performance summaries returned to participants. The scheme covers detection of pathogens, indicator and spoilage organisms and enumeration of specific and total microflora. The isolation rates for the 13 samples distributed during the first year of operation included *Staph. aureus* 94-98%, *Salmonella* 93 and 85% and *L. monocytogenes* 69 and 88%.

PARTIAL PURIFICATION, CHARACTERIZATION AND POTENTIAL APPLICATIONS OF JENSENIIN G, A BACTERIOCIN PRODUCED BY *PROPIONIBACTERIUM JENSENII* P126

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Jenseniin G is a heat stable bacteriocin produced by *Propionibacterium jensenii* P126. It is active against propionibacteria, lactobacilli, lactococci, and streptococci. The inhibitor(s) of propionibacteria and lactobacilli in crude jenseniin G was active over the range of pH 3.0 to 11.0. Jenseniin G retained activity against lactobacilli after suspension in 4M urea or 0.5% SDS. Activity against propionibacteria has not been assessed. The pI of jenseniin G was between 11.0 and 11.5. SDS-PAGE analysis resolved jenseniin G to a single stained band of about 8,500 daltons that with activity against lactobacilli. Initial studies have examined the use of jenseniin G to control the overgrowth of yogurt starter cultures. Jenseniin G inhibited *L. delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* cultures grown in synthetic medium or milk and prevented acidification of milk by both yogurt cultures. The inhibitory and anti-acidification activities resulted from adding jenseniin to either lag or stationary phase cells. Characterization of jenseniin G and its applications will provide much needed information about bacteriocin-mediated antagonism in the industrial propionibacteria and may lead to additional food uses for these organisms.

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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

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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
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3-A Sanitary Standards for Pressure Reducing and Back Pressure Regulating Valves for Milk and Milk Products, Number 64-00 (08-17N)

Formulated by
International Association of Milk, Food and Environmental Sanitarians
United States Public Health Service
The Dairy Industry Committee

It is the purpose of the IAMFES, USPHS, and DIC in connection with the development of the 3-A Sanitary Standards program to allow and encourage full freedom for inventive genius or new developments. Pressure reducing and back pressure regulating valves specifications heretofore or hereafter developed which so differ in design, material, and fabrication, or otherwise as not to conform with the following standards but which, in the manufacturer's or fabricator's opinion are equivalent or better may be submitted for the joint consideration of IAMFES, USPHS, and DIC at any time.

A

SCOPE

A.1

These standards cover the sanitary aspects of pressure reducing and back pressure regulating valves used on processing equipment and on equipment and lines which hold or convey milk or milk products.

A.2

In order to conform to these 3-A Sanitary Standards, pressure reducing and back pressure regulating valves shall comply with the following design, material and fabrication criteria.

B

DEFINITIONS

B.1

Product: Shall mean milk and milk production.

B.2

Back Pressure Regulating Valve: Shall mean a device which controls product inlet pressure by responding to changes of the inlet pressure by means of a self-acting actuator.

B.3

Pressure Reducing Regulating Valve: Shall mean a device which controls product outlet pressure by responding to outlet pressure changes by means of a self-acting actuator.

B.4

Self-Acting Actuator: Shall mean the device that reciprocates the plug and stem assembly on and off the valve seat within the valve body by means of product, cleaning or sterilization fluid forces within the valve body acting against a diaphragm or series of diaphragms separating the internal and external pneumatic or mechanical forces.

B.5

Diaphragm Neutral Position: Shall mean the position and shape the diaphragm assumes with relationship to the valve body and the valve stem when there are no forces being exerted on either side of the diaphragm.

B.6

Bonnet: Shall mean the chamber on the exterior side of the diaphragm.

B.7

Surfaces

B.7.1

Product Contact Surfaces: Shall mean all surfaces which are exposed to the product and surfaces from which liquids may drain, drop or be drawn into the product.

B.7.2

Nonproduct Contact Surfaces: Shall mean all other exposed surfaces.

B.8

Mechanical Cleaning or Mechanically Cleaned: Shall denote cleaning, solely by circulation and/or flowing chemical detergent solutions and water rinses onto and over the surfaces to be cleaned, by mechanical means.

C

MATERIALS

C.1

All product contact surfaces shall be of stainless steel of the AISI 300 Series*1 or corresponding ACI*2 types (See Appendix, Section E.) or metal which under conditions of intended use is equally corrosion resistant as stainless steel of the foregoing types, and is nontoxic and nonabsorbent, except that:

C.1.1

Rubber and rubber-like materials may be used for gaskets, O-rings, seals, valve seats, valve plugs, diaphragms and parts having the same functional purposes.

C.1.2

Rubber and rubber-like materials when used for the above specified applications shall comply with the applicable provisions of the current 3-A Sanitary Stan-

*1 *The data for this series are contained in the AISI Steel Products Manual, Stainless & Heat Resisting Steels, November 1990, Table 2-1, pp. 17-20. Available from the Iron and Steel Society, 410 Commonwealth Drive, Warrendale, PA 15086 (412-776-9460) (Use current edition/revision).*

*2 *Steel Founders Society of America, Cast Metal Federation Bldg., 455 State St., Des Plaines, IL 60016 (708-299-9160).*

dards for Rubber and Rubber-Like Materials Used as Product Contact Surfaces in Dairy Equipment, Number 18-00.

C.1.3

Plastic materials may be used for gaskets, O-rings, seals, valve seats, valve plugs, diaphragms and parts having the same functional purposes.

C.1.4

Plastic materials when used for the above specified applications shall comply with the applicable provisions of the current 3-A Sanitary Standards for Multiple-Use Plastic Materials Used as Product Contact Surfaces for Dairy Equipment, Number 20-18 as amended.

C.1.5

Rubber and rubber-like materials and plastic materials having product contact surfaces shall be of such composition as to retain their surface and conformational characteristics when exposed to the conditions encountered in the environment of intended use and in cleaning and bactericidal treatment or sterilization.

C.1.6

Rubber and rubber-like materials and plastic materials having product contact surfaces that are a bonded coating or a covering shall be of such a composition as to retain their surface and conformation characteristics when exposed to the conditions encountered in the environment of intended use and in cleaning and bactericidal treatment or sterilization.

C.1.7

The final bond and residual adhesive, if used, on bonded rubber and rubber-like materials and bonded plastic materials shall be nontoxic.[*3]

C.1.8

Diaphragms, O-rings and valve seats may also be made of hard rubber (a vulcanized rubber having a ratio of combined sulfur to rubber hydrocarbon in excess of 15 percent and a Shore A Durometer value in excess of 90) that is nontoxic and relatively resistant to abrasion, will maintain its original characteristics such as form, shape and dimensions and will not affect the product and when subjected to the test regimen set forth in the current 3-A Standards for Plastic Materials Used as Product Contact Surfaces for Dairy Equipment, Number 20-18 as amended, shall (a) comply with the criteria in Section I (1) and Section I (3), (b) have maximum weight gains as set forth in Section I (2) of 0.30 in the Cleanability Response, 0.30 in Product Treatment with Solution I and 0.30 in Product Treatment with Solution J.

C.1.9

In a processing system to be sterilized by heat and operated at a temperature of 250 degrees F (121 C) or higher, all materials having a product contact surface(s) used in the construction of pressure reducing and back pressure regulating valves and nonmetallic component parts shall be such that they can be (1) sterilized by saturated steam or water under pressure (at least 15.3 psig or 106 kPa) at a temperature of at least 250 degrees F (121 C) and (2) operated at the temperature required for processing.

C.2

All nonproduct contact surfaces shall be of corrosion-resistant material or material that is rendered corrosion resistant. If coated, the coating used shall adhere. All nonproduct contact surfaces shall be relatively nonabsorbent durable and cleanable. Parts removable for cleaning having both product contact and nonproduct contact surfaces shall not be painted.

D

FABRICATION

D.1

All product contact surfaces shall have a finish at least as smooth as a No. 4 ground finish on stainless steel sheets, and be free of imperfections such as pits, folds and crevices in the final fabricated form. (See Appendix Section F.)

D.2

All permanent joints in metallic product contact surfaces shall be continuously welded. Welded areas on product contact surfaces shall be at least as smooth as a No. 4 ground finish on stainless steel sheets, and be free of imperfections such as pits, folds, and crevices.

D.3

Appurtenances having product contact surfaces shall be removable for cleaning using simple hand tools used by operating or cleaning personnel, or they shall be cleanable in place and accessible for inspection employing simple hand tools used by operating or cleaning personnel.

D.4

Pressure reducing and back pressure regulating valves that are to be mechanically cleaned shall be designed so that the product contact surfaces of the internal valve body, diaphragm and plug, and all nonremovable components thereto can be mechanically cleaned and are easily accessible for inspection.

D.5

Product contact surfaces not designed to be mechanically cleaned shall be accessible for cleaning and inspection when in an assembled position or when removed. Removable parts shall be demountable using simple hand tools used by operating or cleaning personnel.

D.6

Pressure reducing or back pressure regulating valves to be used in a processing system to be sterilized or those to be mechanically cleaned shall comply with the following:

D.6.1

Due to the inherent tendency to close or to regulate and restrict flow under certain pressure conditions, pressure reducing and back pressure regulating valves to be used in such a system must be designed so that they can be easily maintained fully open either by a mechanical device or by pneumatic forces without removal from the processing system.

[*3] *Adhesives shall comply with 21 CFR Part 175 - Indirect food additives: Adhesives and components of coatings. For sale by Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402 (202-783-3238) (Use current edition/revision, temperature required for processing.*

D.6.2

Where heat sterilization is used, the construction shall be such that all product contact surfaces can be (1) sterilized by saturated steam or water under pressure (at least 15.3 psig or 106 kPa) at a temperature of at least 250 degrees F (121 degrees C) and (2) operate at the temperature required for processing.

D.6.3

Where steam or other sterilizing medium is used, the connection(s) on the inlet and outlet shall be such that the steam lines or other sterilizing medium lines can be securely fastened to the pressure reducing and back pressure regulating valves.

D.7

All product contact surfaces shall be self-draining when the regulating valve is properly installed and the valve is in the open position.

D.8

All sanitary fittings and connections shall conform with the applicable provisions of the current 3-A Sanitary Standards for Plug-type Valves for Milk and Milk Products, Number 51-00.

D.9

Gaskets

D.9.1

Gaskets having a product contact surface shall be removable or bonded.

D.9.2

Bonded rubber and rubber-like materials and bonded plastic materials having product contact surfaces shall be bonded in a manner that the bond is continuous and mechanically sound so that when exposed to the conditions encountered in the environment of intended use and in cleaning and bactericidal treatment or sterilization, and the rubber and rubber-like material or the plastic material does not separate from the base material to which it is bonded.

D.9.3

Grooves in gaskets shall be no deeper than their width, unless the gasket is readily removable and reversible for cleaning.

D.9.4

Gasket grooves or gasket retaining grooves in product contact surfaces for removable gaskets shall not exceed 1/4 in. (6 mm) in depth or be less than 1/4 in. (6 mm) wide except those for standard O-rings smaller than 1/4 in. (6 mm), and those provided for in Section D.8.

D.10

Radii

D.10.1

All internal angles of 135 degrees or less on product contact surfaces shall have radii of not less than 1/8 in. (3 mm) except that:

D.10.1.1

Smaller radii may be used when they are required for essential functional reasons, such as those in the bonnet-body connection and the plug guide. In no case shall such radii be less than 1/32 in. (1 mm), except that:

D.10.1.1.1

When for functional reasons the radius must be less than 1/32 in. (1 mm), in such applications as flat sealing surfaces and flow control apertures, the product contact surface of these internal angles must be readily accessible for cleaning and inspection.

D.10.1.2

The radii in gasket grooves, gasket retaining grooves or grooves in gaskets, shall be not less than 1/8 in. (3 mm) except for those for standard 1/4 in. (6 mm) and smaller O-rings, and those provided for in D.8.

D.10.1.3

The radii in grooves for standard 1/4 in. (6 mm) O-rings shall not be less than 3/32 in. (2 mm) and for standard 1/8 in. (3 mm) O-rings shall be not less than 1/32 in. (1 mm).

D.11

DIAPHRAGM CLAMPING POINTS

D.11.1

When the diaphragm is in its neutral position, the angle formed between the diaphragm and the wall of the valve body, at the clamping point on the product side, shall be not less than 90 degrees.

D.11.2

When the diaphragm is in its neutral position, the angle formed between the diaphragm and the stem attachment, at the clamping point on the product side, shall be not less than 90 degrees. This requirement pertaining to the clamping point is not applicable if the stem attachment is completely encapsulated by the diaphragm material.

D.11.3

The clamping point(s) on the diaphragm shall be designed so that there is effective liquid sealing at the clamping point(s) regardless of the valve stroke position of the diaphragm.

D.12

There shall be no threads on product contact surfaces.

D.13

Pressure reducing and back pressure regulating valves shall not have stuffing boxes.

D.14

The bonnet shall be secured to the body with a minimum number of clamps or bolts. The diaphragm shall separate the product from the nonproduct contact surfaces within the bonnet.

D.15

Visual detection of leakage caused by a ruptured diaphragm shall be provided by one of the following means:

D.15.1

On pressure reducing and back pressure regulating valves that utilize a mechanical self-actuating actuator and a single diaphragm to separate the product from the working assembly in the bonnet, the bonnet shall have one or more 3/32 in. (2 mm) holes just above the bonnet flange in a suitable area(s) located so that one hole will be at the lowest point in the installed position for the detection of leakage.

D.15.2

On pressure reducing and back pressure regulating valves that utilize a pneumatic self-actuator and two

diaphragms to separate the product from the nonproduct area, the neutral area between the diaphragms shall have one or more 3/32 in. (2 mm) holes between the diaphragms in a suitable area(s) located so that one hole will be at the lowest point in the installed position for the detection of leakage, and vent to the outside when the retaining ring is in place.

D.16

Interior surfaces of the valve bonnet and cover shall be readily accessible for cleaning and inspection. The valve bonnet and all bonnet parts shall be readily demountable.

D.17

Nonproduct contact surfaces shall have a smooth finish, free of pockets and crevices, and be readily cleanable and those surfaces to be coated shall be effectively prepared for coating.

APPENDIX

E

STAINLESS STEEL MATERIALS

Stainless steel conforming to the applicable composition ranges established by AISI for wrought products, or by ACI for cast products, should be considered in compliance with the requirements of Section C.1 herein. Where welding is involved, the carbon content of the stainless steel should not exceed 0.08 percent. The first reference cited in C.1 sets forth the chemical ranges and limits of acceptable stainless steel of the 300 Series. Cast grades of stainless steel corresponding to type 303, 304, 316 and 316L are designated CF-16F, CF-8, CF-8M and CF-3M respectively. The chemical composition of these cast grades are covered by ASTM*[4] specifications A351/A351M, A743/A743M, and A744/A744M.

F

PRODUCT CONTACT SURFACE FINISH

Surface finish equivalent to 150 grit or better as obtained with silicon carbide, properly applied on stainless steel sheets is considered in compliance with the requirements of D.1 herein. A maximum Ra of 32 micro in. (0.80 micro m), when measured according to the recommendations in ANSI/ASME B46.1 - Surface Texture is considered equivalent to a No. 4 finish.*[5]

G

INFORMATION PLATE

G.1

Manufacturers should provide an information plate in juxtaposition to the name plate giving the following information or the information should appear on the nameplate: MANUFACTURER, MODEL, PRESSURE/TEMPERATURE RATING, SIZE, SPRING-PRESSURE RANGE.

G.2

All identification or information plate(s) affixed to regulating valves should be attached in such a way as to be effectively sealed.

H

DIAGRAMS

These diagrams are intended to demonstrate general principles only, and are not intended to limit individual ingenuity. The design used should conform with the sanitary requirements set forth in these 3-A Sanitary Standards. The following examples are included in this Appendix:

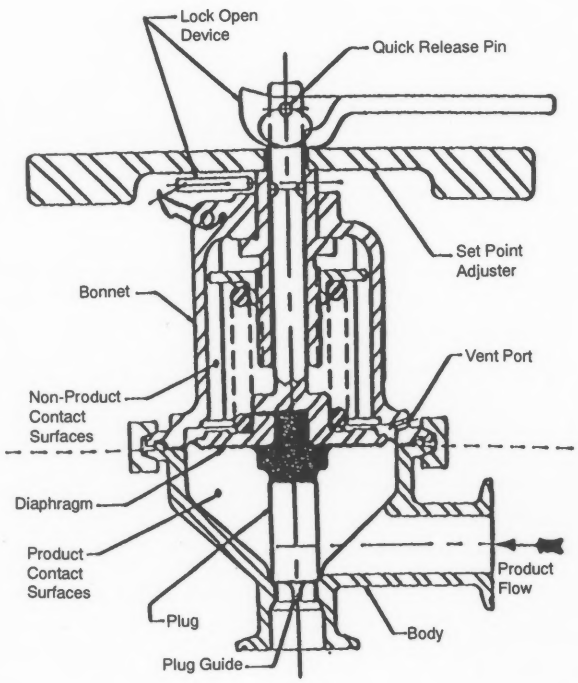
Valve Name	Page	3-A Drawing Number
Back Pressure Regulating Valves	616	3-A-6400-01
Pressure Reducing Regulating Valves	617	3-A-6400-02
Pressure Reducing/Back Pressure Regulating Valves	617	3-A-6400-03

[4] Available from ASTM, 1916 Race St., Philadelphia, PA 19103-1187 (215-299-5400) (Use current edition/revision).

[5] Available from the American Society of Mechanical Engineers, 345 E. 47th Street, New York, NY 10017-2392 (212-705-7722) (Use current edition/revision).

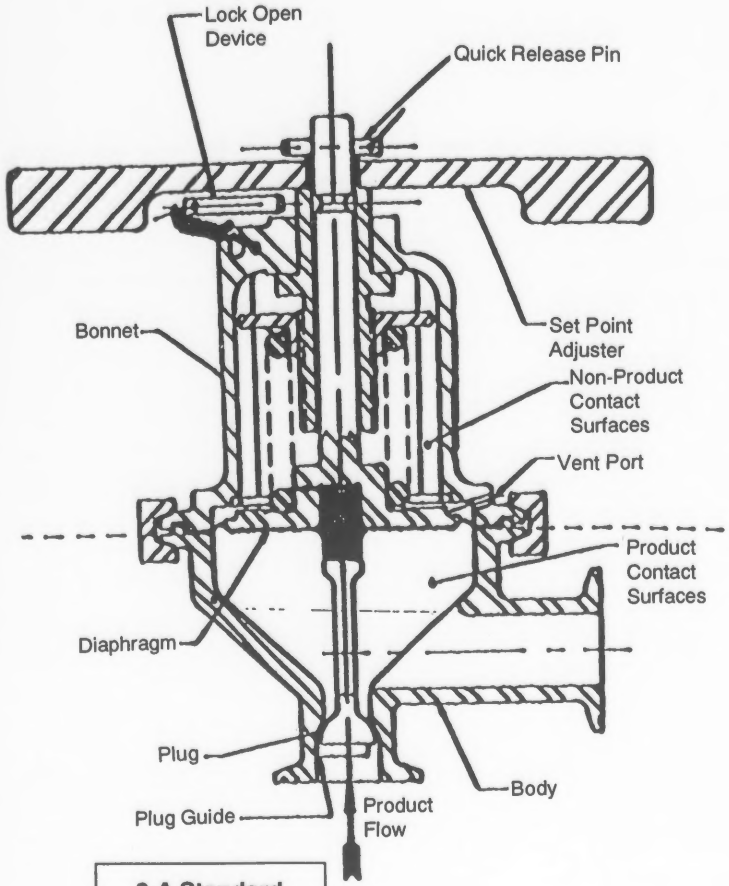
These standards will become effective November 21, 1993.

BACK PRESSURE REGULATING VALVE



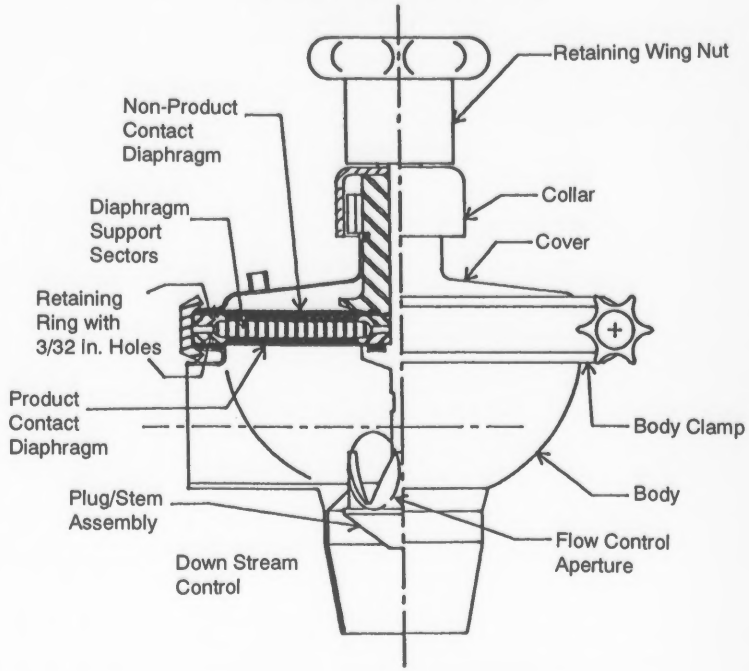
3-A Standard Sanitary Fittings 3-A-6400-01

PRESSURE REDUCING REGULATING VALVE

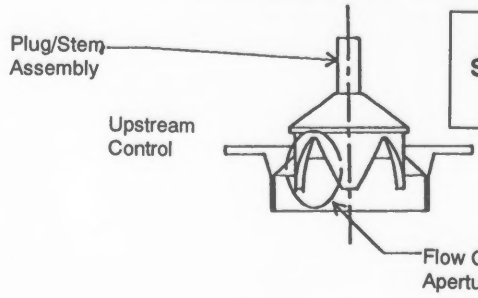


3-A Standard Sanitary Fittings 3-A-6400-02

PRESSURE REDUCING REGULATING VALVE



BACK PRESSURE REGULATING VALVE



3-A Standard Sanitary Fittings 3-A-6400-03

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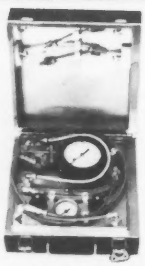

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Coming Events

1993

November

•2-3, **North Dakota Environmental Health Association's Annual Meeting** to be held at the Doublewood in Bismarck, ND. For more information, contact Garry Hoffman, ND Department of Agriculture at (701)224-4763.

•3, **HACCP Traing and Certification Workshop for the Seafood Industry**, to be held at the Southern California Gas Company, Downey, CA. Co-sponsored by the University of California Sea Grant Extension Program, the National Fisheries Institute, and the California Fisheries and Seafood Institute. Registration fee: $100. For more information contact Robert Price (916/752-2194) or Pamela Tom (916/752-3837), Food Science and Technology Department, University of California, Davis, CA 95616 (FAX: 926/752-4759).

•3-5, **Gum Chemistry and Technology**, sponsored by the American Association of Cereal Chemists, will be held in Chicago, IL. For more information, contact Marie McHenry, AACC Short Course Coordinator, 3340 Pilot Knob Road, St. Paul, MN 55121-2097, USA. Telephone: (612)454-7250; FAX (612)454-0766.

•5, **Food Labels: Learning the New Language**, A Workshop on the New FDA and USDA Food Labeling Requirements, will be held in Dallas, TX (downtown). This workshop is co-sponsored by The American Dietetic Association Foundation and The Food Processors Institute and developed with a grant from Campbell Soup Company. For more information contact The Food Processors Institute (DLC), 1401 New York Avenue, NW, Suite 400, Washington, DC 20005, (202)393-0890.

•6, **Food Labels: Learning the New Language**, A Workshop on the New FDA and USDA Food Labeling Requirements, will be held in Dallas, TX (suburbs). This workshop is co-sponsored by The American Dietetic Association Foundation and The Food Processors Institute and developed with a grant from Campbell Soup Company. For more information contact The Food Processors Institute (DLC), 1401 New York Avenue, NW, Suite 400, Washington, DC 20005, (202)393-0890.

•10-11, **Alabama Association of Milk, Food and Environmental Sanitarians** will hold their Annual Meeting at the Howard Johnson, Montgomery. For more information contact Dr. Tom McCaskey at (205)844-1518.

•10-13, **National Training Conference** sponsored by the National Society for Healthcare Foodservice Management will be held at the Greenbrier Resort, White Sulphur Springs, WV. For further information and registration details, contact HFM at (202)546-7236.

•12, **Food Labels: Learning the New Language**, A Workshop on the New FDA and USDA Food Labeling Requirements, will be held in New York, NY. This workshop is co-sponsored by The American Dietetic Association Foundation and The Food Processors Institute and developed with a grant from Campbell Soup Company. For more information contact The Food Processors Institute (DLC), 1401 New York Avenue, NW, Suite 400, Washington, DC 20005, (202)393-0890.

•13, **Food Labels: Learning the New Language**, A Workshop on the New FDA and USDA Food Labeling Requirements, will be held in Northern New Jersey. This workshop is co-sponsored by The American Dietetic Association Foundation and The Food Processors Institute and developed with a grant from Campbell Soup Company. For more information contact The Food Processors Institute (DLC), 1401 New York Avenue, NW, Suite 400, Washington, DC 20005, (202)393-0890.

•14-16, **The Food Industry Environmental Conference and Exhibition**, presented by the Environmental Science and Technology Laboratory and Georgia Tech Research Institute, will be held at the Omni Hotel at CNN Center, Atlanta, GA. For more information contact Edd Valentine or Charles Ross at (404)894-3806.

•15-17, **Pennsylvania Association of Dairy Sanitarians and Dairy Laboratory Analysts Fall Meeting** will be held at Penn State University, University Park, PA. For more information, contact Mike John at (717)762-7789.

•16-18, **Food Extrusion**, sponsored by the American Association of Cereal Chemists, will be held in Sydney, Australia. For more information, contact Marie McHenry, AACC Short Course Coordinator, 3340 Pilot Knob Road, St. Paul, MN 55121-2097, USA. Telephone: (612)454-7250; FAX (612)454-0766.

•17, **Ontario Food Protection Association Annual Meeting** will be held at the Valhalla Inn, Etobicoke, Ontario, Canada. For more information call Debbie Labelle at (519)885-8741 or Anna Lammerding at (519)822-3300.

•18, **Food Labels: Learning the New Language**, A Workshop on the New FDA and USDA Food Labeling Requirements, will be held in Washington, DC. This workshop is co-sponsored by The American Dietetic Association Foundation and The Food Processors Institute and developed with a grant from Campbell Soup Company. For more information contact The Food Processors Institute (DLC), 1401 New York Avenue, NW, Suite 400, Washington, DC 20005, (202)393-0890.

•19, **Food Labels: Learning the New Language**, A Workshop on the New FDA and USDA Food Labeling Requirements, will be held in Philadelphia, PA. This workshop is co-sponsored by The American Dietetic Association Foundation and The Food Processors Institute and developed with a grant from Campbell Soup Company. For more information contact The Food Processors Institute (DLC), 1401 New York Avenue, NW, Suite 400, Washington, DC 20005, (202)393-0890.

•29-Dec. 2, **Better Process Control School**. For more information please contact Robert Price (916/752-2194) or Pamela Tom (916/752-3837), Food Science and Technology Department, University of California, Davis, CA 95616-8598, FAX: 926/752-4759.

December

•**2-3, Starch: Structure, Properties, and Food Uses**, sponsored by the American Association of Cereal Chemists, will be held in Chicago, IL. For more information, contact Marie McHenry, AACC Short Course Coordinator, 3340 Pilot Knob Road, St. Paul, MN 55121-2097, USA. Telephone: (612)454-7250; FAX (612)454-0766.

•**3, Food Labels: Learning the New Language**, A Workshop on the New FDA and USDA Food Labeling Requirements, will be held in Kansas City, MO. This workshop is co-sponsored by The American Dietetic Association Foundation and The Food Processors Institute and developed with a grant from Campbell Soup Company. For more information contact The Food Processors Institute (DLC), 1401 New York Avenue, NW, Suite 400, Washington, DC 20005, (202)393-0890.

•**5, Food Labels: Learning the New Language**, A Workshop on the New FDA and USDA Food Labeling Requirements, will be held in Cleveland, OH. This workshop is co-sponsored by The American Dietetic Association Foundation and The Food Processors Institute and developed with a grant from Campbell Soup Company. For more information contact The Food Processors Institute (DLC), 1401 New York Avenue, NW, Suite 400, Washington, DC 20005, (202)393-0890.

•**6-7, Food Safety for Zero Defects Seminar**, sponsored by ASI Food Safety Consultants, Inc., will be held at the Holiday Inn O'Hare International, Chicago, IL. For more information, call Kim Schroeder at (800)477-0778 or in Missouri at (314)725-2555.

•**8-10, Symposium on Antibiotics and Sulfonamides in Milk: Significance, Detection and Development of an Integrated Detection System**, sponsored by the International Dairy Federation with AOAC International, to be held at the Conference Centre Kolle Kolle, Copenhagen, Denmark. For more information contact Prof. Dr. W. Heeschen, Bundesanstalt fur Milchforschung, Hermann Weigmann-Str.1, 2300 Kiel 1, Germany, Tel. +49 431 609 392, FAX: +49 431 609 222.

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January

•**3-5, Milling for Cereal Chemists**, sponsored by the American Association of Cereal Chemists, will be held in Manhattan, KS. For more information, contact Marie McHenry, AACC Short Course Coordinator, 3340 Pilot Knob Road, St. Paul, MN 55121-2097, USA. Telephone: (612)454-7250; FAX (612)454-0766.

•**25-28, Water Activity: Theory, Management, and Food Applications**, sponsored by the American Association of Cereal Chemists, will be held in St. Paul, MN. For more information, contact Marie McHenry, AACC Short Course Coordinator, 3340 Pilot Knob Road, St. Paul, MN 55121-2097, USA. Telephone: (612)454-7250; FAX (612)454-0766.

March

•**7-10, Better Process Control School**. For more information please contact Robert Price (916/752-2194) or Pamela Tom (916/752-3837), Food Science and Technology Department, University of California, Davis, CA 95616-8598, FAX: 926/752-4759.

•**16, Annual Food Industry Conference** will be sponsored by the Food Science Department at Purdue University. For more information contact James V. Chambers, Food Science Department, Smith Hall, Purdue University, West Lafayette, IN 47907, Phone: (317)494-8279.

April

•**18-21, Purdue Better Process Control School** will be sponsored by the Food Science Department at Purdue University. For more information contact James V. Chambers, Food Science Department, Smith Hall, Purdue University, West Lafayette, IN 47907, Phone: (317)494-8279.

May

•**7-12, Food Structure Annual Meeting** will be held at the Holiday Inn Downtown City Hall, Toronto, Ontario, Canada. For more information, please contact Dr. Om Johari, SMI, Chicago (AMF O'Hare), IL 60666-0507, USA (or call 708-529-6677, FAX: 708-980-6698).

August

•**31-August 3, 81st Annual Meeting of the International Association of Milk, Food and Environmental Sanitarians** will be held at the Hyatt Regency Hotel, San Antonio, TX. For more information contact: Julie Heim — Registration; Scott Wells — Exhibits; at (800)369-6337 (US), (800)284-6336 (Canada), or (515)276-3344.

To insure that your meeting time is published, send announcements at least 90 days in advance to: IAMFES, 200W Merle Hay Centre, 6200 Aurora Avenue, Des Moines, IA 50322.

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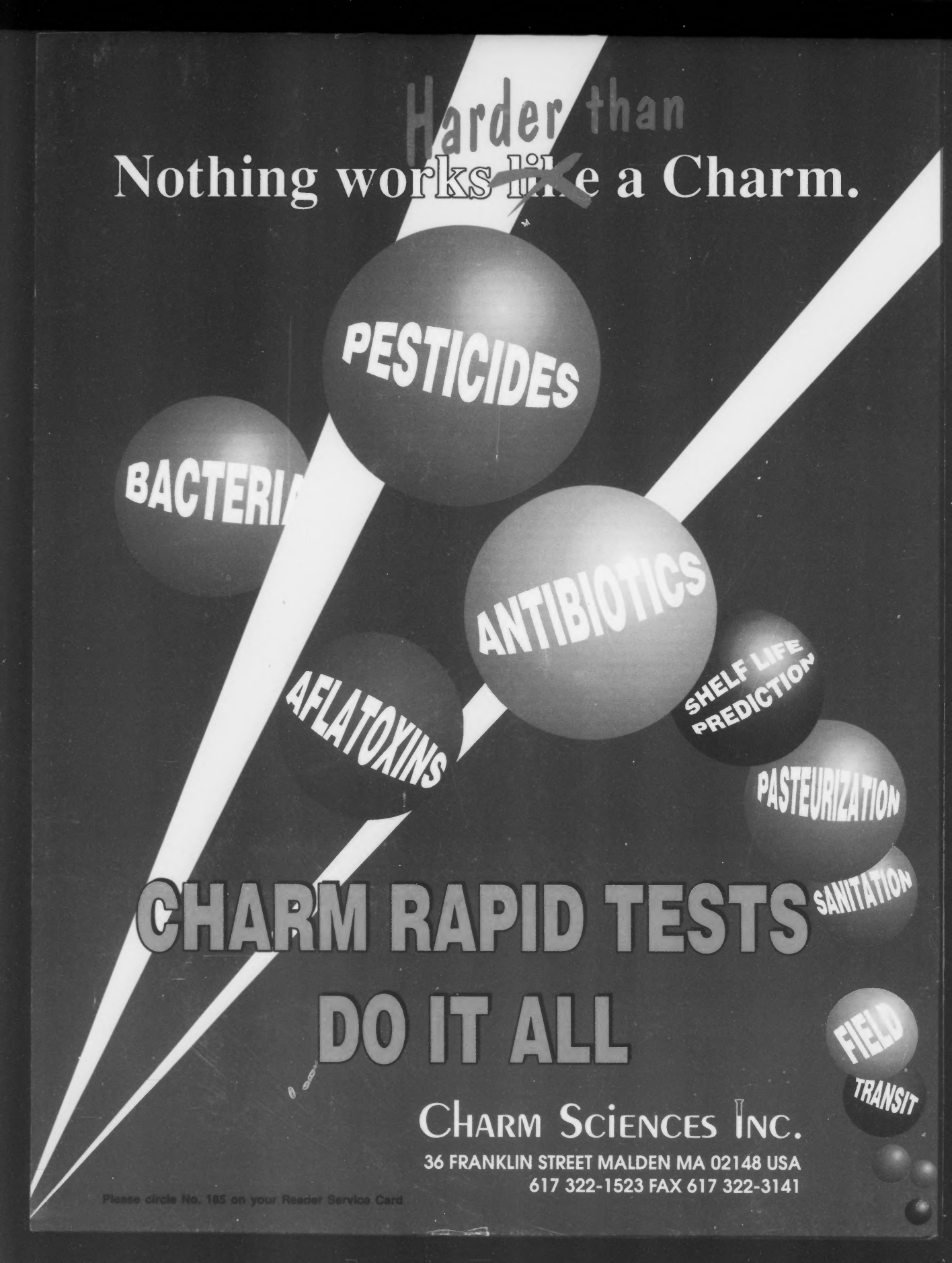
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