

## MICROBIAL RISK MANAGEMENT IN FOOD PROCESSES

2<sup>nd</sup> Workshop arranged by SAFOODNET – Food Safety and Hygiene Networking within New Member States and Associated Candidate Countries



VTT SYMPOSIUM 260

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**SAFOODNET – FOOD SAFETY AND HYGIENE NETWORKING  
WITHIN NEW MEMBER STATES AND ASSOCIATED CANDIDATE  
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## PREFACE

Food Safety and Hygiene Networking within New EU Member States and Associated Candidate Countries (SAFOODNET FP6-022808) is a specific support action EU-project building-up a food safety network. It aims at knowledge sharing to prevent risks related to microbial hazards, to find future RTD needs and apply for RTD funding in food processing and packaging safety. The action focuses towards Czech Republic, Denmark, Estonia, Finland, Hungary, Latvia, Slovenia and Turkey in the pilot actions, seminars, and workshops on process hygiene and end product safety. Interested researchers and SME representatives from other new EU countries and ACCs are encouraged to participate in the activities. The objectives of SAFOODNET are to: 1) disseminate knowledge from national and international food safety projects in open seminars, workshops, practical exercises, RTD activities and pilot actions resulting in new research projects for food industry especially SMEs; 2) establish an expert group (EG) in which authorities, scientists, industrial representatives build-up or strengthen existing networks and identify specific needs for future RTD activities in food safety and 3) bridge networks within the new EU, fostering scientific co-operation and knowledge transfer in food safety.

The workshop on *Microbiological risk management in food processes* focused on preventive activities such as Good Management Practice (GMP), equipment design, surface materials and factory layout. It has been shown that obtaining representative samples in the process industry is a demanding task, because microbes causing problems can be tightly attached to the surfaces or the process equipment may contain dead ends or bending in pipes that are hard to access. Furthermore, the conditions during sample transportation have great impact on quality of results obtained. This 3-day long workshop with both the theoretical and the practical sessions on *Microbial risk management* focused on the follow-up of raw material quality in food processing including packaging materials and lubricants, implementation and monitoring of critical control points of the process, corrective actions, documentation and content of internal hygiene audits. For more information see the project homepage <http://safoodnet.vtt.fi>.



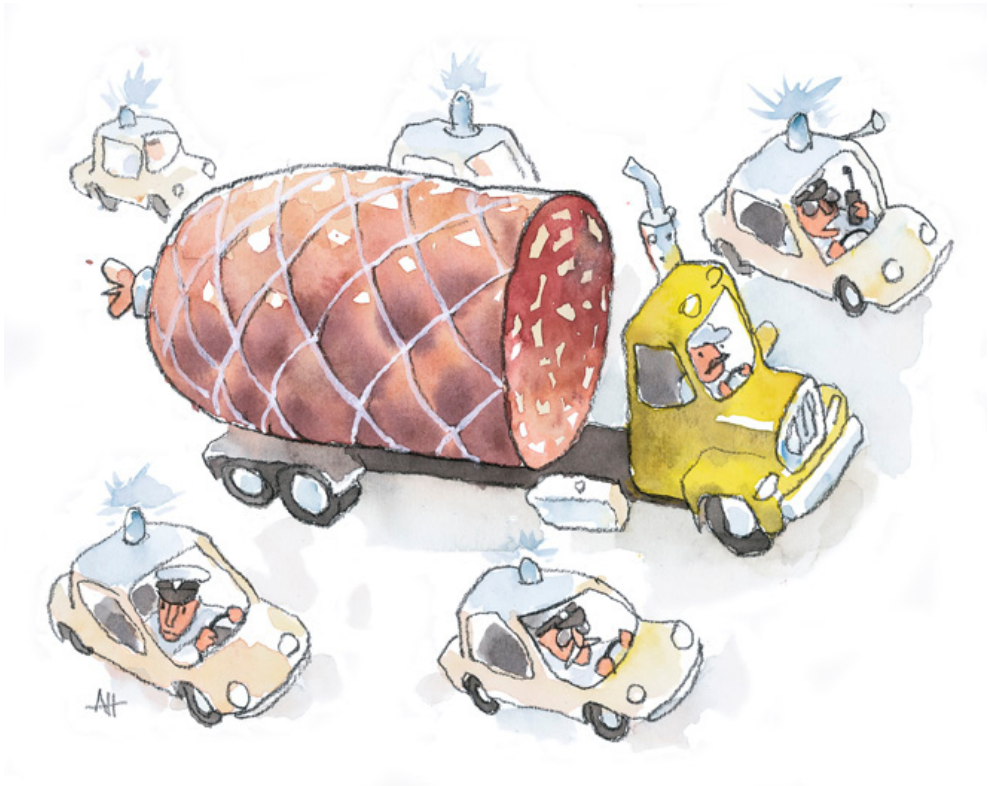
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# INFLUENCE OF FLUID FLOW AND CFD IN CLOSED PROCESSES

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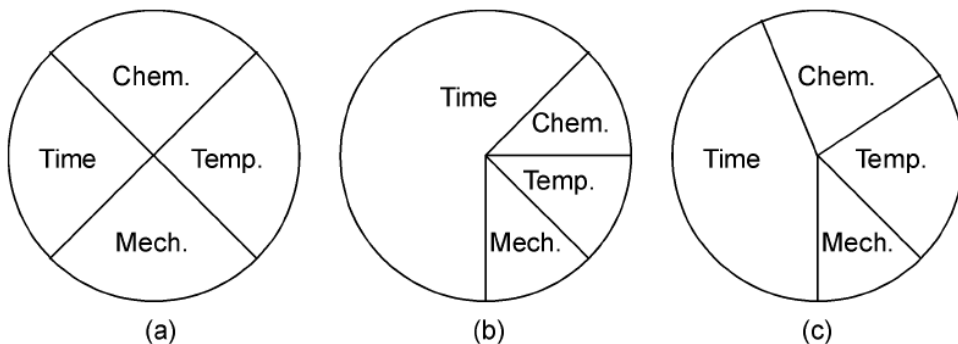
\*now Alfa Laval Tank Equipment

Good hygienic performance of closed processing equipment for food processing depends on aspects such as the construction material, the surface finish (roughness and topography), gaskets material and design, welding quality, cleaning procedure (time, temperature and detergent) and the physical design of the equipment. This presentation focuses on another important relationship namely the relation between the fluid flow of detergent during cleaning and the physical design of the equipment. Maintenance of proper hygiene in closed process equipment is in many ways a complex task. The interaction between the design of the equipment and the nature of fluid flow in the equipment is the main concern. It is already known that dead legs or other types of areas shielded from the main flow can occur and present a hygienic risk. The nature of the flow is crucial since the flow is responsible for bringing cleaning fluid and heat to the process surface and subsequent removal of residues which are cleaned of the surfaces. The focus is not on the straight pipes and other relatively easy to clean parts of process lines but on the more complicated parts where there is a risk of having a much reduced impact of the fluid flow.

The impact of fluid flow is evaluated using the wall shear stress and the fluid exchange both as separate parameters and the action of the two in combination. This is based on a basic understanding of the interaction between the flow characteristics and soil attached to surfaces. Such information can be obtained from fluid dynamics theory and models or by rules of thumb and be used to assist improvement the design of process equipment with respect to cleaning characteristics and optimisation of cleaning procedures. Prediction of cleaning efficiency in especially complex parts of closed process plants by use of computational fluid dynamics (CFD) is an excellent tool for desktop improvements

and serves a possibility for computer pre-validation of the hygienic performance of process plants. Hence, the hygienic design of closed equipment related to the movement of detergent can be improved.

Proper hygienic design of closed equipment is an exercise of making detergent (temperature and chemicals) accessible to the soil for a certain period (time) and exposing the soil to a force (mechanical) that is sufficiently large to remove the soil from the surface. Sinner (1960) suggests that cleaning efficiency is a combination of four cleaning parameters temperature, chemicals, time and mechanical action. A change in one of the parameters in Sinner's circle must be compensated for by changes in the three other parameters. Additionally, detergent and heat have to be transported to the soil on the surface to be effective and the soil must be removed from the surface and out of the equipment to avoid reattachment. In this section, the importance of flow is illustrated based on Sinner's circle and the effect of flow on the four parts of Sinner's circle is introduced.



The influence of the transport phenomenon on cleaning time illustrated by Sinner's circle for (a) a fully developed turbulent pipe flow, (b) a recirculation zone in, e.g., a dead-end with poor exchange of detergent and (c) a recirculation zone in, e.g., a valve with a good exchange of detergent.

Any cleaning procedure can be considered as a process of applying the energy required to remove soil from a surface. The energy may according to Sinner be divided into four sources: contact time, detergent temperature, detergent action and mechanical action. The temperature and chemicals weaken the bond between soil and surface as a function of temperature, strength of chemicals and contact time between detergent and soil. The contact time is the time a specified temperature and strength of detergent are present at the interface between soil and detergent.

The mechanical effect is the shear force acting on soil at the surface. The shear force removes the soil from the surface. When cleaning closed equipment, all four of the components of Sinner's circle are influenced, either directly or indirectly, by the flow of detergent inside the equipment. The direct influence is through the mechanical force acting on the soil. The force is generated from motion of the detergent across the surface. The force is also known as the wall shear stress.

The influence of flow on contact time is best illustrated by comparing two common situations. In a straight pipe detergent flows parallel to the wall and 'fresh' detergent (temperature and chemistry) is continuously transported across the soil, resulting in an ideal cleaning conditions concerning the cleaning time (illustrated by situation (a) in the figure above). In a dead-end or a sudden change of geometry, flow recirculation is present. In such recirculation zones, the detergent is not replaced at the same rate as in the straight pipe i.e. both heat and mass transfer rates are low and the temperature and strength of the detergent decrease slightly as a function of time. Hence, the contact time compared to the straight pipe is reduced and the total cleaning time is increased for surfaces located in the recirculation zone (illustrated in the figure above situation (b) and (c)).

We are using CFD simulations of the flow inside closed process equipment to visualise fluid flow and make a relation to the cleaning action based on two parameters wall shear stress and fluid exchange. Research has proven that these parameters can explain a great part of the cleaning action of fluid flow. The investigation is based on both experimental data and generally accepted mechanisms of cleaning, that data regarding the flow conditions obtained using CFD could in fact be used for explaining why certain areas of different types of equipment were difficult to clean and others were not. We have shown that the outcome of the EHEDG cleaning test, described in the guideline number 2 "A method for assessing the in-place cleanability of food processing equipment" ([www.ehedg.org](http://www.ehedg.org)) can be predicted from CFD simulations visualising wall shear stress and fluid exchange. The steps needed to make a prediction of the areas with different degrees of cleanability are: 1. a critical wall shear stress under controlled flow conditions is needed for the cleaning test method; 2. wall shear stress and fluid exchange is predicted using a CFD model of the piece of equipment; 3. areas exposed to different levels of wall shear stress in relation to the critical value are identified  $\pm$  a rough estimation of cleanability is possible; 4. areas exposed to different levels of fluid exchange relatively to the fluid exchange in the undisturbed

part of the flow are identified; 5. grouping the different areas of wall shear stress and fluid exchange makes the prediction of areas of different cleanability possible.

It is possible to manufacture closed processing equipment to optimise the design of equipment with respect to the cleaning effect of fluid flow. This should lead to equipment with a better hygienic design than is seen today. Comparing data from cleaning tests with information on flow patterns obtained from CFD simulations also aids in obtaining a more thorough understanding of certain flow patterns, positive or negative, on the cleaning efficiency. Furthermore, data from CFD simulations could be used to visualise areas of potential hygiene problems in closed processing equipment. This would make it clear to non-specialists in hygienic design and fluid dynamics why certain areas are problematic because of unfavourable flow conditions.

# INDUSTRIAL AND ANALYTICAL METHODS FOR THE DETECTION OF INDUSTRIAL FOOD FOULING

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In an industrial plant where continual cleaning and soiling occurs the detection of residual organic soil and cells is necessary to monitor surface hygiene. Build up of organic material on surfaces may result in a potential nutrient source for pathogens and food soil microorganisms, pathogen transfer of microbial cells and subsequent food contamination. A range of rapid industrial and analytical methods were used to compare the detection of residual cells and soil on stainless steel. Rapid industrial methods included ATP (adenosine triphosphate) bioluminescence and an ultra violet (UV) light detection method. Microscopy *in vitro* methods included Scanning Electron Microscopy (SEM), and epifluorescence microscopy. Methods used to detect changes in the surface physicochemistry included contact angle, surface free energy, dispersive and polar measurements, whilst chemical methods included Energy Dispersive X-ray (EDX) and Fourier Transform Infrared Spectroscopy (FTIR).

Organic soils included complex (meat extract, fish extract, cottage cheese extract) and defined soils (oils [cholesterol, fish oil, mixed fatty acids]; proteins [bovine serum albumin, fish peptones casein]; carbohydrates [glycogen, starch, lactose]); at a range of concentrations (10% to 0.001%). Rapid industrial methods used for the detection of residual cells (*Listeria monocytogenes* or *Escherichia coli*) and soil e.g. ATP (adenosine triphosphate) bioluminescence and a UV light detection method were assessed for their ability to detect organic soils, or organic soil-cell mix on surfaces. Under UV, oily soils, mixed fatty acids, cholesterol and casein were detected at low concentrations, with detection levels ranging from 1% to 0.001% for different substances. Glycogen was the most difficult substance to detect at lower concentrations. Using 330–380 nm, 510–560 nm and 590–650 nm wavelengths different results were obtained. A wavelength of 330–380 nm illuminated most of the soils well, whilst 510–560 nm illuminated the fish extract, cholesterol and fatty

acids; the 590–650 nm wavelength illuminated the lactose. Soils at all concentrations were detected by the ATP bioluminescence method, the complex soils giving the highest readings. When complex soils were combined with *L. monocytogenes* Scott A or a non pathogenic *E. coli* 0157:H7, ATP measurements increased by 1–2 logs. For UV illumination, the *L. monocytogenes* on cheese was the most intensely illuminated, with *E. coli* in meat the least.

Scanning electron microscopy (SEM) and epifluorescence microscopy were used to visualise the soil and its distribution across the surface. Using epifluorescence microscopy the percentage of a field covered by soil was also accessed via image analysis. At 10% concentration, most soils, with the exception of BSA and fish peptone were easily visualised using SEM, presenting differences in gross soil morphology and distribution. When soil was stained with acridine orange and visualized by epifluorescence microscopy, the limit of detection of the method varied between soils, but some (meat, cheese and glycogen) were detected at the lowest concentrations used (0.001%). Overall complex soils were detected at the lowest concentrations using epifluorescence microscopy whilst proteins needed to be at a high concentration. At higher concentrations of soil, SEM is a useful qualitative tool to examine the gross morphology and distribution of some food soils once dried onto a surface. Epifluorescence microscopy provides a simple and quantitative method for determining the pattern of distribution and surface coverage by the soils. The decrease in soil concentration applied to the surface did not relate directly to the percentage coverage measurement.

Physicochemical parameters (surface hydrophobicity, surface free energy (SFE), polar and dispersive measurements) were used to determine alterations in the surface following fouling with organic materials. At higher concentrations, soiling surfaces with complex soils, proteins and carbohydrates generally made surfaces more hydrophilic, with increased SFE and polar measurements, and decreased dispersive measurements. Oil soils increased surface hydrophobicity and lowered SFE, polar and dispersive measurements. Changes in surface physicochemistry were not directly related to the concentration of soil applied to the surface. Results indicate that under certain conditions, contact angle and polar measurements could be used to detect higher concentrations of surface fouling which may not be immediately apparent to the naked eye. However, data does not reveal the food group soil type present on the surface.



Energy dispersive X-Ray (EDX) and Fourier Transform Infra Red Spectroscopy (FTIR) were used to determine the detection levels, and/or components of a range of organic food soils on stainless steel surfaces. It was shown that using percentage carbon, EDX would be useful for the detection of organic material on surfaces where oily based residues predominate. FTIR could identify specific peaks related to biochemical groups (oils, proteins, carbohydrates) on the surfaces. Spectra for the complex materials and the oils were varied whereas spectra for the proteins and carbohydrates were similar within their groups. Both the techniques tested require sophisticated equipment and trained personnel. Samples for analysis using EDX needed extensive preparation whereas FTIR samples required sufficient knowledge for the elucidation of results.

This work helps to understanding of the nature of organic soil fouling on surfaces in the food industry. UV illumination is a simple well established method for detecting significant food soil by eye, with little change in findings when microbes are included. Performance can be enhanced in certain circumstances by altering the wavelength. ATP bioluminescence is a proven system for hygienic assessment being especially useful in the presence of microbes rather than soil alone.

Microscopy methods such as epifluorescence and SEM allow the distribution of most of the soils to be visualised; Epifluorescence is especially useful at lower concentrations of soil. However care needs to be taken since some soils such as BSA were not detected at higher concentrations using SEM. Physicochemical methods were useful for detecting general trends when surfaces were fouled with soil. However data were not easy to interpret; this may be since this method uses measurements on the micron scale whereas the changes in surface properties may be taking place on a molecular scale, and surface fouling takes place over a macro scale. Thus it may be suggested that this is not the best method to use to determine organic fouling in an industrial system.

The EDX and FTIR methods were suitable for more in depth analysis of organic material retained on surfaces and would be useful to use in conjunction with other methods described to determine the nature of residual fouling left on surfaces following cleaning. This might facilitate the development of the best cleaners for use in particular industries. However no one method detected all the soils, or gave easily identifiable data for groups of soils.

The use of a range of detection methods demonstrated that a variety of techniques need to be used if the effect and type of food soil retained on a surface is to be elucidated. Clearly some of these techniques are less suitable for use on site in the food industry but supporting data enhances the simpler methods leading to improved cleaning techniques. The detection and identification of retained soil is of importance since retained soil will affect the cleanability of the surface and thus the retention of microbes and possible pathogens. This series of work compared each of these methods to assess their merits, shortfalls and relationships in terms of soil detection.

# MICROBIAL ISSUES IN FISH FACTORIES – HOW TO USE DISINFECTION ?

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The microflora of fish is to some extent a reflection of the microflora in their environment. Hence, the microflora of fish from colder waters is likely to be psychrotrophic in nature and very different from warm blooded animals. However, during further processing, seafood processed in modern fish industry is exposed to many of the same potential risks in terms of contamination as any other food product.

The level of human pathogenic bacteria in the general environment and in fish is generally low, however, it is important, to realise that several potentially human pathogenic bacteria are a natural part of the aquatic environment. In example *Listeria monocytogenes* and *Clostridium botulinum* are found in the general environment and can hence be detected on fish and shellfish. Fish caught in coastal areas may carry pathogenic bacteria from the animal/human reservoir due to contamination of this aquatic environment for example *Salmonella* spp., *Shigella* spp., and *E. coli* are associated with faecal contamination of seafood. Furthermore contamination can occur during production, i.e. contamination with *Staphylococcus aureus*, *Salmonella* spp. or other pathogens may occur during manual handling of cooked products, such as shrimps and crustaceans. In the same way unless processing equipment in fish slaughter houses are adequately maintained from a sanitary perspective, contamination problems by plant persistent bacteria like *L. monocytogenes* may occur.

*L. monocytogenes* is commonly isolated from food production plants, including fish slaughterhouses and fish smokehouses. Therefore this talk will focus on control of *L. monocytogenes* in the processing environment as an important example. Products such as cold-smoked salmon must be considered high risk products as such products support growth of *L. monocytogenes*. No critical control points can be identified for *L. monocytogenes* in cold-smoked salmon production because the process parameters

used such as temperature and salt concentration do not eliminate the bacteria. Furthermore classical preservation parameters do not guarantee against growth. Targeted prerequisite programs are needed and only a reduction, but not elimination can be obtained by good manufacturing practice.

By a targeted sample collection, with the purpose of finding *L. monocytogenes*, a relatively high occurrence between 16 to 50% during production and 9 to 27% after sanitization was found in four smokehouses producing cold-smoked fish and four fish slaughterhouses. Thus, in general, the hygiene in the plants could be improved. Different contamination patterns were detected in the plants. In smokehouse 1 several different *L. monocytogenes* sub-types were identified, in smokehouse 2 a dominant, persistent subtype was present, in smokehouse 3 two dominating subtypes were found, and in smokehouse 4 a persistent sub-type was found only outside the raw fish area. Some sub-types were found in more smoke-/slaughterhouses, especially one sub-type was frequently occurring. This sub-type may occur very frequently in nature, however, it is more likely that this sub-type is especially strong in colonizing process equipment, or in out-competing other subtypes. However it is still not known why some types persist and some do not.

The talks discusses the concept of niches which are specific areas in which *L. monocytogenes* persists and disinfection processes, which can effectively eliminate niches that are already established. We have recently demonstrated that an important factor allowing *L. monocytogenes* to persist could be its remarkable ability to withstand drying conditions as it under particular conditions is capable of surviving on surfaces for up to three months. It is extremely important to prevent establishment of *L. monocytogenes* as an in-house flora that can be a continuous source of contamination. The principles behind a *Listeria* control program is describes as a continuous surveillance of *L. monocytogenes* occurrence in the process environment. *Listeria* reduction can only be obtained by increased focus on hygiene and cleaning and disinfection procedures.

On-going research at our laboratory focuses on evaluation and development of new physically based disinfection procedures. Also, we work with optimisation of conventional disinfection procedures. Physically based disinfection processes are unlikely to cause development of tolerance or resistance in the target microorganisms. The talk will outline the principles of ozone technology, UV-C-light and the newly developed combination of hot steam and ultrasound (SonoSteam).

# TESTING OF DISINFECTION EFFICACY

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Disinfection is required in food plant operations where wet surfaces provide favourable conditions for the growth of microbes. An effective disinfection minimises contamination of the product, enhances product shelf-life and reduces the risks of foodborne illness. Disinfectants approved for use in the food industry contain alcohols, oxidants, iodophor- and chlorine-based compounds, persulphates, surfactants and quaternary ammonium compounds. Requirements set in the Biocidal Products Directive 98/8/EC (BPD) will in the near future change the approval procedure of disinfectants for use in food industry, which is one of the 23 product types described in the BPD. Technical Notes for Guidance on preparation of dossiers for placing biocidal products on the market has been launched to facilitate collection of Summary Dossiers under the BPD. These dossiers must include copies of original tests and study summaries on active substances and biocidal products, risk assessment of active substances and biocidal products and risk characterisation as well as an overall summary. The new approval system will most probably decrease the number of active agents on the market.

Today the efficacy of disinfectants and antimicrobial agents are usually still determined in suspensions with free cell. This do not mimic the growth conditions of microbes growing in biofilms on surfaces, where the agents are required to inactivate the microbes. At VTT the activity of disinfectants has been tested using the Dutch 555-suspension test protocol or its modification to find out the bactericidal, fungicidal and sporicidal activity of the disinfectant. In this test as in many of the below mentioned standards the activity is measure after a challenging period of 5 min. In the 555-test the product is efficient in suspension when the microbial reduction is at least 5 log-units for vegetative cells and the product shows sporicidal activity when the reduction of spores is at least 1 log-unit. The microbes commonly used in the 555-test are *Salmonella Choleraesuis*, *P. aeruginosa*, *S. aureus*, *B. cereus* (spores) and *S. cerevisiae*. The test is carried out using bovine albumin as organic load. In a modified 555-suspension test the disinfectant is tested

against a chosen panel of process contaminants (consisting of bacteria, yeasts and/or moulds) in bovine albumin for 5–30 min periods at temperatures of interest. It has been shown that a prolongation of the disinfection enhances the microbicidal effect on items and surfaces. A prolonged exposure is recommended especially if there is problems with bacterial spores or fungal cells or if the agent is used at low temperatures. The testing protocols standardised by the European Committee for Standardization (CEN) varies depending on the contamination source studied. CEN has recently updated many of the disinfectant test standards and it has also launched new ones with for studying the bactericidal, sporicidal, yeasticial, fungicidal and viricidal activities, e.g. the following standards:

- in EN 1040:2005 the basic bactericidal activity of a disinfectant is quantitatively tested in suspension against both Gram-negative (*Pseudomonas aeruginosa* ATCC 15442) and Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538),
- in EN 14347:2005 the basic sporicidal activity of a disinfectant is quantitatively tested in suspension against spores of *Bacillus subtilis* subsp. *spizizenii* ATCC 6633 and *Bacillus cereus* ATCC 12826,
- in EN 1275:2005 the basic fungicidal activity of a disinfectant is quantitatively tested in suspension against both yeast and mould strains (*Candida albicans* ATCC 10231 & *Aspergillus niger* ATCC 16404 as spores),
- in EN 1656:2000 the bactericidal activity of a disinfectant for use in veterinary area is quantitatively tested in suspension against both Gram-negative and Gram-positive bacteria (*P. aeruginosa* ATCC 15442, *E. coli* ATCC 10536, *S. aureus* ATCC 6538 and *Enterococcus hirae* ATCC 10541) in hard water with organic load,
- in EN 14476A1:2006 the viricidal activity of a disinfectant for use in human medicine is quantitatively tested in suspension against Poliovirus and Adenovirus in organic soil for instruments and surfaces as well as in buffered peptone saline for hygienic handwash and handrub products as well as Parvovirus in organic soil for chemothermal disinfection, and
- in EN 13704:2002 the sporicidal activity of a disinfectant is quantitatively tested in suspension against spores of *B. subtilis* ATCC 6633 (and if necessary *B. cereus* ATCC 12826 and *Clostridium sporogenes* CIP 7939) for use in food, industrial, domestic and institutional areas in hard water suspension with low organic load.

Furthermore, there are standards for testing the bactericidal, fungicidal and yeasticidal activities of disinfectants using carriers for instrument disinfection and using nonporous surfaces without mechanical action for surface disinfection in the veterinary area as well as standard procedures for studying the basic activity of hygienic handwash and handrub products.

The standard suspension tests have proven to be reliable, because the variations of results are within acceptable limits in the replications of tests. The efficacy testing performed only using suspension tests is not enough to show that an agent is effective, because the agent must also be efficient on surfaces. In test with surface-attached microbes the agent should reduce the microbial level with at least 3–4 log units in order to be considered effective. At the moment there is only one standardised protocol for testing disinfectant efficacy on surfaces and it covers efficacy testing of disinfectants for use in the veterinary area (EN 14349:2007). When planning a protocol for testing the disinfectant efficacy on surfaces it is important to identify the major sources of variation in the procedure, so that the deviation in the test results is as small as possible. The following factors e.g. carrier material, organic soil, viability of dried cells and reproducibility of biofilm growth on the surfaces affect the procedure. Microbes growing or dried on surfaces are furthermore not susceptible to disinfectants from all sides as they are in suspensions. The disinfectants must often be used in higher concentrations on surfaces than in suspensions to be effective.

# COMMERCIAL DISINFECTANTS AND THEIR APPLICATIONS

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Looking back in history we can find disinfectants which are still in use today. Let take a look at the most used groups of disinfectants:

- Hypochlorite or chlorine -based disinfectants were the first widely used disinfectants in the dairy/beverage/brewery and food industry. They are effective against many microorganisms, but are potentially corrosive and face increasing environmental restrictions.
- The iodine containing disinfectants have limited activity against spores, can stain equipment surfaces, and can taint products.
- The hypochlorites, along with iodine, are halogen disinfectants. Many companies have moved away from halogen disinfectants for several reasons, including:
  - Corrosion of stainless steel equipment
  - Concerns about halogen residuals in food products
  - Concerns about halogen effluent
  - Ineffectiveness of some halogens when heavy soiling is present.
- Quaternary-ammonia disinfectants or quats are non-corrosive, but have limited antimicrobial activity. Quats are strongly foaming, which limits their usefulness in CIP applications.
- Acid and acid anionic disinfectants are effective against most bacteria and some yeast, but not spores. They were developed for CIP and spray applications. They typically also contain high levels of phosphate which, in view of more demanding environmental restrictions, becoming less and less accepted in our industries. Some disinfectants, based on recently discovered synergies between selected organic fatty acids, were successfully introduced to the market within the last few years.



- Peracetic acid disinfectants represent a new chemistry and provide outstanding antimicrobial activity against most bacteria, but have limited activity against a number of yeast, especially wild strains. They are effective across a broad pH and temperature range.

In mixed peracid disinfectants a combination of peracids enables P3-oxysan generation technology to be used at much lower concentration than peroxyacetic acid alone, with improved yeast, mould, and spore antimicrobial activity. Lower pH of the use solution may help reduce mineral film build-up. Lower hydrogen peroxide levels minimise the potential for oxidation of sensitive beverage products.

Disinfectants are applied in many different applications and their working mechanism must be considered before choosing a disinfectant. Applications for disinfectants are in circulating (closed systems), foaming, spraying, fogging and long contact disinfection (dipping bath).

The disinfectants can be divided into two groups – oxidative disinfectants and stable disinfectants. Oxidative acting disinfectants such as hypochlorite, hydrogen peroxide, peroxy acetic acid, iodine etc has the following working mechanism: Their antimicrobial effect is caused by oxidation that irreversibly destroys biologically active systems of the cell. The differences in efficacy depend largely on the different oxidation potentials. As an acid that is little dissociated, peroxy acetic acid does not only react with proteins of the cell wall, but also penetrates into the inner part of the cell. Cleaning prior to disinfection is particularly important with these product types and the overall application recommendation is on surfaces in closed systems (CIP).

The stable, non reactive disinfectants such as QAC, biguanide, amines, glucoprotamin etc is working by perforation of cell wall/membrane. Changes on the cell wall disturb its permeability and the cell content can be released to the environment causing the death of the cell. For the stable types of products the final rinse is particularly important and the overall application recommendation is usage on production surroundings.

An example from the oxidising group is peroxy acetic acid (POAA), which is one of the most used types of disinfectants in the food industry especially in dairy and beverage industry. POAA is highly efficient in killing all kinds of microorganisms,

including yeast, mould and bacteriophages and spore forming bacteria. POAA is also known as one of the most effective disinfectants against biofilms. Other advantages of POAA is: low or no foam, a combined disinfection and acid rinse, no or low residues and an environmentally responsible approach as its contains no phosphor and the active ingredients are broken down to water and oxygen.

From the group of stabile, non reactive disinfectants we find fatty amines. They are cationic surfactants and very similar to quaternary ammonium compounds in their way of action. Fatty amine acetate are used as foaming disinfectant (P3-topax 99) for open surfaces and combines the detergent effect (lower surface tension) with microbial effect. Fatty amines (in combination with nonionic surfactants and fatty acid derivates) are also used as chain lubricants in the beverage industry, where they reduce friction on chain conveyors. The conveyors are used for bottle transporting and besides the lubrication effect the amines also eliminates biofilm on the chains.

# FOOD SAFETY IN PRACTICE

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Food safety is of fundamental importance to the consumers, the food industry and the economy. The food industry can attack food safety in different ways e.g. in a proactive way or in a reactive way. Proactive steps in the production plan can be implemented by making a plan for preventive inspections and to observe and react. Bactoforce is an independent service provider which helps companies in the pharmaceutical, biochemical and food industries with the recognition and prevention of microbiological risks in the production plan. Bactoforce has the philosophy that today's action can prevent tomorrow's hazards and it is better to be safe than sorry. If this motto applies anywhere, then it is definitely in the pharmaceutical, biochemical and food industries. Here are several inspections described which is a part of the services that Bactoforce offers to companies.

**HygieneSafe** is a general inspection of complete process installations and production processes for microbiological risks and inventory of general hygiene conditions are important. Scratches, hairline cracks or other irregularities in process installations can lead to early or late-developing of micro-biological problems. However, it is often just a case of small defects which are un- or barely perceptible with the naked eye. In practice, action is only taken if measurable micro-biological anomalies appear. Micro-biological risks can be detected in their early stages. For example, Bactoforce is able to literally bring 'invisible' irregularities to light by using UV light. Bactoforce also looks for more deep-set causes of micro-biological risks. For example, it is quite possible that damage to the process installation is the result of the production or cleaning methods used.

**TankSafe** can be used for inspection of tanks and spray dryers for deposits and microcracks (crack tests) as well as CIP validation. Micro-biological risks are often in small nooks. This can for example be in blind spots in tanks or spray dryers or in small imperfections such as hairline cracks. However, often the cleaning method used is the cause of micro-biological irregularities. Therefore, the tank or spray dryer should

be inspected and also the (CIP) cleaning process. Bactoforce does this for tanks and spray dryers of all sorts and sizes from small tanks in the biochemical industry to fermentation tanks of more than 500,000 litres in the beer industry. Bactoforce has developed a unique inspection method for large tanks and spray dryers which allows us to inspect the entire surface without having to use scaffolding.

**PastSafe** can be used in inspection of plate-type heat exchangers and in measurement of heating times in pasteurisers and UHT installations. From the viewpoint of efficiency, plate-type heat exchangers are a godsend for the food industry. However, they can entail some risks. The smallest imperfection in the thin plates can lead to the mixing of treated and untreated products, with all the consequent effects. It is therefore important regularly to carry out preventative checks on the heat exchangers. Bactoforce has developed a method whereby we can test plate-type heat exchangers on location, in a closed state, for leakages, hairline cracks and other defects and also localise diagnosed defects especially closely. In doing so, we save our clients enormous time and expense. Not just because of machine downtimes but also because no more new seals have to be put in place. In addition, Bactoforce also carry out inspections of heating times in pasteurisers and UHT installations. The methods Bactoforce uses for this allow us to determine parameters such as pressure and temperature extremely accurately, which facilitates the prevention of future micro-biological risks.

**PipeSafe** is a video-endoscopic inspection for bad weld-joints and other defects or risks in pipe systems can be done. Bad weld-joints in pipe systems constitute a large hygiene risk for process installations. Hairline cracks and other imperfections which can easily remain undetected in piping also occur regularly. Bactoforce makes use of an especially efficient method in order to inspect the pipe system for the presence of these sorts of risks: video-endoscopy. This allows us to detect the causes of micro-biological irregularities as quickly as possible or to determine the current condition of your pipe work. With regards to prevention, Bactoforce also often carry out video-endoscopic inspections during the assembly of new process installations in order to map all possible risks before the production begins. Video-endoscopy is also extremely appropriate for the inspection of complicated pipe systems. Systems are often just inspected from the viewpoint of the condition of the equipment, for weld-joints for example; product remnants can easily get left behind. Think of places where measurement apparatus are installed in the pipe work, or 'dangerous' areas in the construction.

# PREDICTIVE MICROBIOLOGY AND APPLICATION OF MATHEMATICAL MODELS

Paw Dalgaard

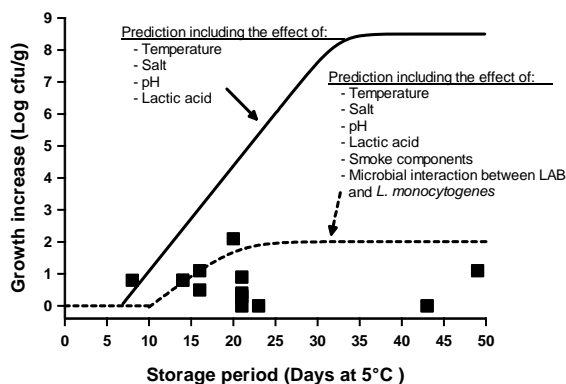
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Growth of micro-organisms is of major importance for safety and shelf-life of numerous fresh and lightly preserved foods. Consequently, the growth of both human pathogens and spoilage micro-organisms must be evaluated. These evaluations are particularly important when products are formulated, re-formulated and when packaging or conditions in the food chain are changed, including storage and all steps of distribution to the consumer. Challenge tests, with inoculated products, and storage trials, with naturally contaminated food, are important tools for evaluations of safety and spoilage. Both challenge tests and storage trials, however, are time consuming, labour demanding, costly and usefulness of the results obtained is limited to the specific product characteristics and storage conditions studied. Clearly, it is interesting to predict growth of pathogenic and spoilage micro-organisms in food depending on processing (heat, high pressure, freezing, etc.), product characteristics (salt/ $a_w$ , pH, organic acids, smoke components, competing microflora/bacteriocins, etc.) and storage conditions (temperature, atmosphere and humidity). Such predictions allow safety and shelf-life of food to be evaluated rapidly for a range of constant or changing processing, product and storage conditions. Mathematical models of microbial growth are particularly important for risk-based food safety assessment and management as they relate food safety objectives (FSOs), performance objectives (POs) and performance criteria (PC). To efficiently support decisions on food safety and quality management, predictions must be relatively simple to obtain and reasonably accurate. If, for example, a product actually has a safe shelf-life of 30–40 d at 5°C then a predicted shelf-life of 3–4 d at 5°C (one order of magnitude less) will, in most situations, be misleading rather than supportive of good management decisions.

## PREDICTIVE MICROBIOLOGY

Modelling of microbial growth in food relies on basic microbial kinetics and it is part of the food microbiology sub-discipline called predictive microbiology. Growth of micro-organisms in food is reproducible. Consequently, mathematical models that quantitatively describes the combined effect of important environmental parameters can be used to predict microbial growth and thereby product safety or shelf-life.

Figure 1. Observed and predicted growth of *Listeria monocytogenes*. The observed growth (■) was determined at 5°C for 13 different lots of naturally contaminated vacuum-packed cold-smoked salmon. Predictions were obtained by using the model of Mejlholm and Dalgaard (J. Food Prot. 70, 2007, 70–85 and J. Food Prot. 70, 2007, 2485–2497). The figure clearly shows the importance of including all relevant environmental parameters in a predictive model.



To make prediction of microbial growth in food the important environmental parameters must be known or identified and their concentration/level must be measured. For fresh food such as milk, meat or fish the important environmental parameters that determine growth of micro-organisms are typically limited to temperature and atmosphere (with pH and naturally occurring lactic acid having an effect for some food/micro-organism combinations). However, for lightly preserved foods such as cheese, smoked meat or marinated fish the situation is more complex. The important environmental parameters can include temperature, atmosphere, salt/ $a_w$ , pH, naturally occurring or added organic acids (e.g. acetic, benzoic, citric, lactic or sorbic acids), smoke components, food structure and microbial interactions/bacteriocins (see Fig. 1).

Simple kinetic models are sufficient to describe and predict growth of micro-organisms in food under the large majority of situations observed in practice. Primary models of microbial growth describe how the concentration of a microorganism ( $N$ , cfu/g) changes over time. These models have a differential form

for the absolute growth rate ( $dN/dt$ , cfu/g/h) and an integrated form to directly describe the concentration of cells at different points in time (Table 1). By comparing model simulations (Figure 2) and the differential forms of the Exponential and the Logistic models it is seen that the Logistic model describes exponential growth when the cell concentration at the time  $t$  ( $N_t$ ) is much lower than  $N_{max}$  and that a growth dampening is described when  $N_t$  approached  $N_{max}$ . The Logistic model has also been expanded with a parameter ‘ $m$ ’ to include a flexible growth dampening ( $dN/dt = N \cdot \mu_{max} \cdot [1 - (N_t / N_{max})^m]$ ) and this model can be relevant for example when growth in food is related to microbial formation of metabolites such as histamine.

Figure 2. Simulation of the Exponential model (dotted line), the Logistic model (dashed line) and the Logistic model with delay (solid line). Table 1 shows the differential and integrated forms of these simple classical kinetic models for microbial growth.

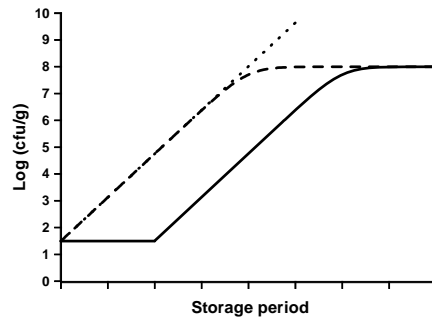


Table 1. Examples of simple and classical kinetic growth models<sup>a</sup>.

Model name	Differential form of model	Intetrated and log-transformed model
Exponential	$\frac{dN}{dt} = N \cdot \mu_{max}$	$\text{Log}(N_t) = \text{Log}(N_0 \cdot \exp(\mu_{max} \cdot t))$ or $\text{Log}(N_t) = \text{Log}(N_0) + (\mu_{max} \cdot t) / \text{Ln}(10)$
Logistic	$\frac{dN}{dt} = N \cdot \mu_{max} \left[ 1 - \frac{N_t}{N_{max}} \right]$	$\text{Log}(N_t) = \text{Log} \left( \frac{N_{max}}{1 + \left[ \frac{N_{max}}{N_0} - 1 \right] \cdot \exp(-\mu_{max} \times t)} \right)$
Logistic with delay	$\frac{dN}{dt} = N \cdot \mu_{max} \left[ 1 - \frac{N_t}{N_{max}} \right], t > t_{lag}$ $\frac{dN}{dt} = N \cdot \mu_{max} \left[ 1 - \frac{N_t}{N_{max}} \right], t > t_{lag}$	$\text{Log}(N_t) = \text{Log}(N_0), t \leq t_{lag}$ $\text{Log}(N_t) = \text{Log} \left( \frac{N_{max}}{1 + \left[ \frac{N_{max}}{N_0} - 1 \right] \cdot \exp(-\mu_{max} \times (t - t_{lag}))} \right), t > t_{lag}$

<sup>a</sup> ‘ $t$ ’ is the time of storage; ‘Log’ is  $\text{Log}_{10}$ ; other parameters are explained in the text

The Logistic model with delay represent at simple way of describing a lag phase (Fig. 2). To estimate the kinetic parameters in this model ( $t_{lag}$ ,  $N_0$ ,  $\mu_{max}$  and  $N_{max}$ ) so called piecewise non-linear regression is required. Concentrations of microorganisms in food are often expressed as  $\text{Log}(N_t)$ , rather than  $N_t$ , for example when growth curves are presented. The Log-transformation results in an approximately constant variance of the concentration data over time and this makes the growth curve “look nice”. To maintain the meaning of the parameters in an integrated primary growth model both the left and the right side of the equation must be log-transformed as shown in Table 1.

Secondary growth models describe the effect of environmental parameters on the key kinetic parameters in primary growth models, often maximum specific growth rate ( $\mu_{max}$ ) and lag time ( $t_{lag}$ ) but probability of growth can also be modelled. Secondary models relying on polynomials, constrained linear polynomials and artificial neural networks have been used extensively within predictive food microbiology. These models, however, will not be further discussed here because very large amounts of experimental data are needed to develop robust secondary models of those types. In contrast, cardinal parameter or square-root type secondary models can be developed from markedly less data as explained below.

Secondary cardinal parameter models (CPMs) and square-root type models rely on the so called gamma ( $\gamma$ )-concept suggested by Marcel Zwietering and colleagues in 1992. The basic idea is to determine the maximum specific growth rate under optimal growth conditions ( $\mu_{max-opt}$ ). This growth rate is then reduced by each of the environmental parameters that differ from the optimal growth condition. The reduction of the growth rate is expressed by a  $\gamma$ -factor that has a value between 0 and 1 for each of the important environmental parameters (Eq. 1 and 2, Fig. 3).

As shown in Fig. 3 and Eq. 2 cardinal parameters models exclusively include parameters with a biological meaning such as  $\mu_{max-opt}$ ,  $T_{min}$ ,  $T_{opt}$ ,  $T_{max}$ ,  $pH_{min}$ ,  $pH_{opt}$  and  $pH_{max}$ . This facilitates the estimation of the parameter values. However, data are needed to estimate growth at for example high temperatures and high pH values and this may not be relevant for safety of a chilled food product. To overcome this problem a less complex type of cardinal parameter models can be used as shown in Figure 4. Here  $\mu_{max-opt}$  is replaced with another  $\mu_{max}$ -value determined at a lower reference temperature ( $\mu_{max-ref}$ ) and the range of the model is limited to sub-optimal temperature, water activity and pH values. These models are sometimes also called



square-root models because  $\mu_{max}$ -data are square-root transformed to stabilize their variance when the model parameters are estimated by fitting using non-linear regression.

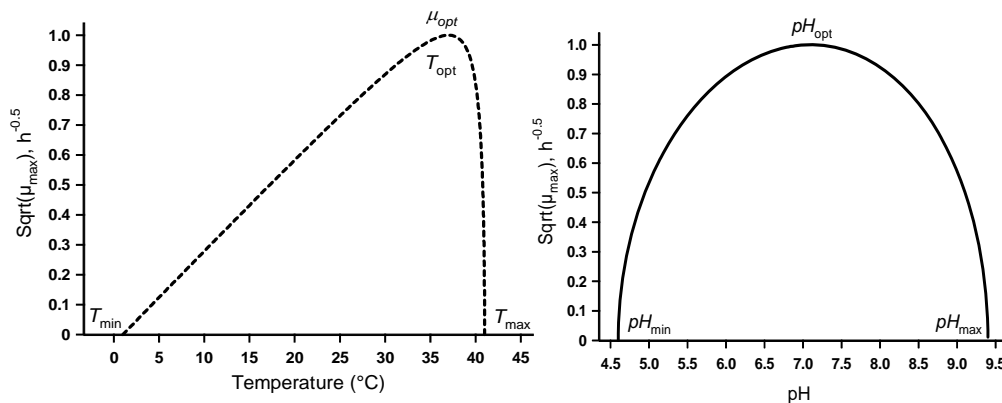


Figure 3. Examples of cardinal parameter models for the effect of temperature and pH on the maximum specific growth rate ( $\mu_{max}$ ) of microorganisms. Curves represent simulations of Eq. 2 with  $n = 2$  for temperature and  $n = 1$  for pH.

$$\mu_{max} = \mu_{max-opt} \cdot \gamma_2(T) \cdot \gamma_1(pH) \cdot \gamma_2(a_w) \cdot \left( 1 - \left( \frac{[\text{Organic acid}]}{\text{MIC}_{\text{Organic acid}}}\right)^{n1} \right)^{n2} \quad (1)$$

$$\gamma_n(X) = \begin{cases} 0, & X \leq X_{min} \\ \frac{(X - X_{max}) \cdot (X - X_{min})^n}{(X_{opt} - X_{min})^{n-1} \cdot [(X_{opt} - X_{min}) \cdot (X - X_{opt}) - (X_{opt} - X_{max}) \cdot ((n-1) \cdot X_{opt} + X_{min} - n \cdot X)]}, & X_{min} < X < X_{max} \\ 0, & X \geq X_{max} \end{cases} \quad (2)$$

Figure 4. Simplified cardinal parameter model. The model includes sub-optimal values of the following seven environmental parameters: Temperature, water activity, pH, concentration of undissociated lactic acid, smoke components/ phenol, carbon dioxide and undissociated acetic acid. The model takes into account interaction between all the environmental parameters and this has been obtained by using the Le Marc approach as described below (J. Food Prot. 70, 2007, 70–85 and J. Food Prot. 70, 2007, 2485–2497).

$$\begin{aligned} \mu_{max} = & \mu_{max-ref} \\ & \cdot \left( \frac{(T - T_{min})}{(T_{ref} - T_{min})} \right)^2 \\ & \cdot \frac{(a_w - a_{w min})}{(a_{w opt} - a_{w min})} \\ & \cdot 1 - 10^{(pH_{min} - pH)} \\ & \cdot 1 - \frac{\text{LAC}_U}{\text{MIC}_{U \text{ LAC}}} \\ & \cdot \frac{(P_{max} - P)}{(P_{max} - P_{opt})} \\ & \cdot \frac{(\text{CO}_2_{max} - \text{CO}_2_{dissolved})}{(\text{CO}_2_{max} - \text{CO}_2_{opt})} \\ & \cdot 1 - \sqrt{\frac{[\text{AC}_U]}{[\text{MIC}_{U \text{ AC}}]}} \\ & \cdot \xi \end{aligned}$$

To be useful in food safety management models of microbial growth must be reasonably accurate and this represents a major challenge. In fact, growth models developed on the basis of growth curves obtained in laboratory experiments with broth can be several fold faster than growth determined in food. Reasons for such deviations include incompleteness so that the effect of one (or more) important environmental parameter is not taken into account. In this situation new terms for individual environmental parameters can be developed from laboratory experiments and added to a model of the type shown in Eq. 1 and in Fig. 4. It is also possible to combine terms from different existing models and this is most important for efficient and cost effective model development.

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# HACCP SYSTEMS

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The Hazard Analysis and Critical Control Point (HACCP) system is based on the recognition that hazardous events may happen at various points during production, and that steps can be taken to control the occurrence of these events. The system offers a rational and logical approach to the control of food safety and avoids the many weaknesses inherent in the inspectional approach (ICMSF 1988). National food legislation authorities are aware of this and in the E.U. full responsibility for the safety of food is now placed on the producers who have to implement a control system based on the principles of HACCP. The HACCP system was developed in the 1960ies for production of safe foods for manned space flights. The American Public Health Association at the National Conference of Food Protection presented it to the public in 1971 (ICMSF 1988). A further development of the system was released by the ICMSF in 1988. The system has since been recognized worldwide as the best system to ensure the safety of foods (Mayes & Mortimore 2001). Application and implementation of HACCP and its principles has been described in details in a number of guidelines (e.g. ICMSF 1988; NACMCF 1992; WHO/FAO 1995; ILSI 1997). In 1997, the outcome of a concerted international attempt to harmonise HACCP principles and terminology was published as a Codex Alimentarius HACCP document (CAC 1997). This document has since been regarded as the international benchmark for effective HACCP implementation (Mortimore & Mayes 2002).

## PRINCIPLES OF HACCP

HACCP should be applied as a systematic approach in the identification of hazards, assessment of their significance and definition of means for their control throughout any production, and/or packaging line at the manufacturing site as well as in the distribution system (CAC 1997). The approach is a stepwise analysis, which is commonly divided in seven principles as outlined in Figure 1. The first principle – conduct a hazard analysis – can be looked upon as the scientific basis for the following managing steps where the decisions are made on how to control the

identified and ranked hazards. The output of the HACCP analysis is a so-called HACCP plan; a document prepared in accordance with the principles to ensure control of any relevant food safety hazards (CAC 1997; ILSI 1997).

## **DEFINITION OF BASIC TERMS**

The terms used within the HACCP system need to be defined before consideration is given to the way the system may be applied. Especially the word *hazard* has to be distinguished from the word *risk*. This is not quite as simple as it may seem, and while it may be tedious it is essential. For instance, according to the dictionary the term 'hazard' is defined as 'risk', which also is reflected in the everyday use of these two words as they are most often used interchangeably. However, within the HACCP system, hazard and risk have their own separate and distinct meaning. Some examples of definitions of these terms found in the literature are given in Table 1.

The definition of the term hazard within the HACCP concept has changed over the years. The most recently published definitions cover chemical, physical, and biological types of hazards that have the potential to cause harm to the consumer (WHO/FAO 1995; CAC 1997), i.e. only the food safety aspect is considered. In contrast, the earlier definitions only covered microbiological hazards but were concerned with both safety and spoilage aspects of the food (ICMSF 1988; Shapton & Shapton 1991). Whether the HACCP system is applicable to both safety and spoilage aspects of food production is still a topic of discussion. However, it is generally believed that safety and spoilage aspects must be addressed separately in order to implement a HACCP system successfully (NACMCF 1992; Mortimore & Mayes 2002).

With regard to the microbiological safety aspect of food another trend in the evolution of the term hazard can be deduced from Table 1. It is the change of the term microbiological hazard from being an event, i.e. contamination, growth, or survival of microorganisms, into being an agent or entity, i.e. the microorganism itself or its toxin(s). The latter definition of hazard has been adopted both in HACCP as well as in the authorities' counterpart, the Risk Analysis concept (Table 1). This evolution of the term hazard also affects the meaning of the term risk. When a hazard previously was regarded as contamination, growth, or survival of microorganisms the risk was the probability of occurrence of these events. In the newest definitions, where a hazard is an agent, the term risk is only used in the context of Risk Analysis – not in HACCP (Table 1).

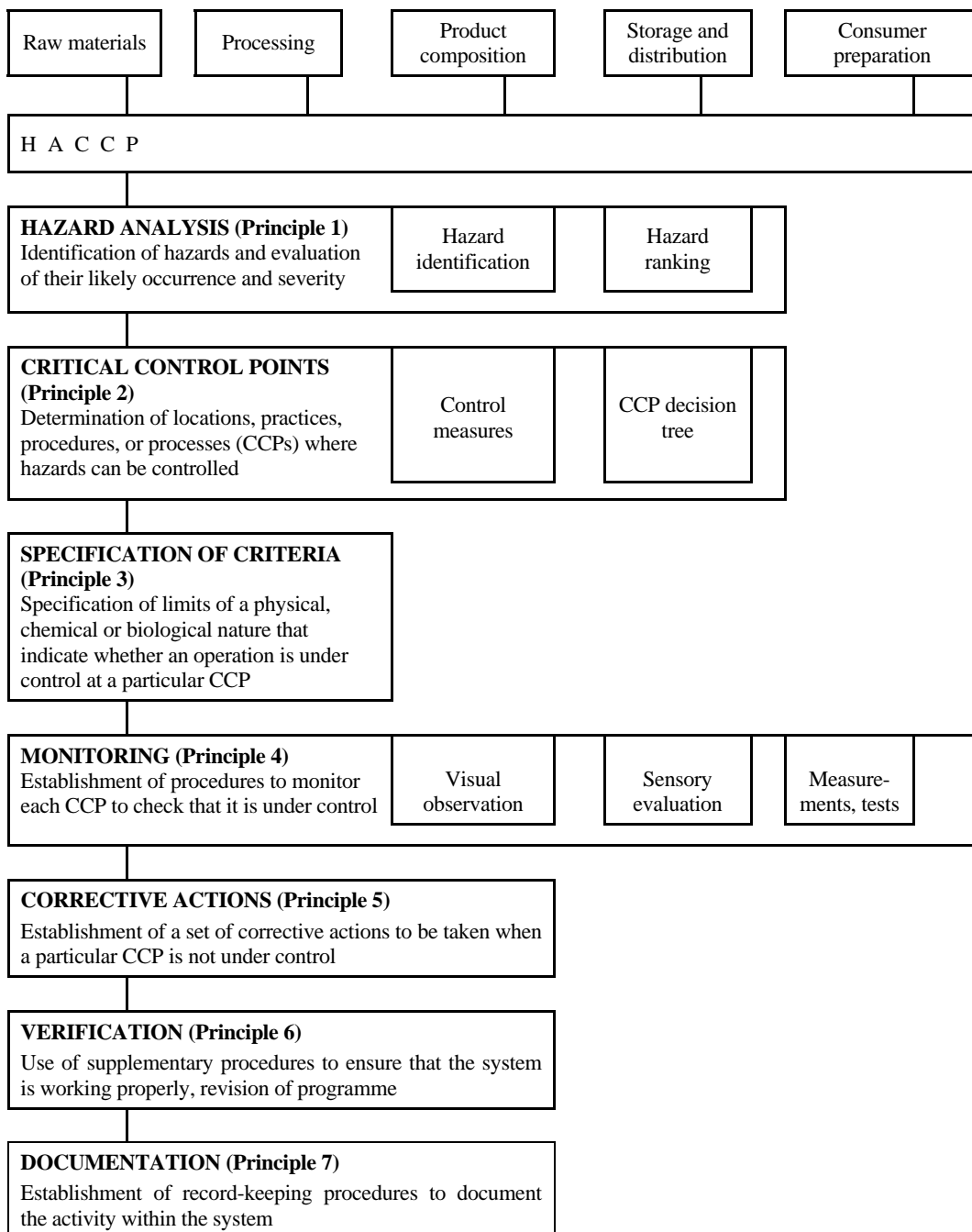


Figure 1. The seven principles underlying every HACCP system (CAC 1997).

In the CAC (1997) HACCP definition it is, however, stated that the likely occurrence of hazards as well as the severity of their adverse health effects should be considered. Severity in relation to Risk Analysis means evaluating the consequences to the consumer (CAC 1996), whereas severity within HACCP may also mean evaluating the consequences to the company if an adverse health effect occurs from consumption of their product. In some way the consequences to the food producing company will be related to the severity of the disease, but other consequences such as product recalls, loss of revenue, and loss of jobs will also have to be considered.

Table 1. Definitions of hazard and risk in relation to food hygiene and safety issues.

<b>Concept</b>	<b>Hazard</b>	<b>Risk</b>	<b>Ref.</b>
HACCP	Unacceptable contamination, growth or survival of bacteria in food that may affect food safety or quality (spoilage) or unacceptable production or persistence in foods of substances such as toxins, enzymes or products of microbial metabolism	An estimate of the probability or likelihood of a hazard occurring	ICMSF (1988)
HACCP	The potential to cause harm to the consumer (safety aspect) or to the product (spoilage aspect) and is present at any stage in the product lifespan where unacceptable microbial contamination or where growth or survival of unwanted microbes may occur	The probability that a hazard will be realized or will happen	Shapton & Shapton (1991)
HACCP	Biological, chemical, or physical property that may cause a food to be unsafe for consumption	An estimate of the likely occurrence of a hazard	NACMCF (1992)
HACCP	Biological, chemical, or physical agent with the potential to cause adverse health effect when present at an unacceptable level	Not used	WHO/FAO (1995)
HACCP	A biological, chemical or physical agent in, or condition of, food with potential to cause an adverse health effect	Not used	CAC (1997)
Risk Analysis	Biological, chemical or physical agent in, or condition of, food with potential to cause an adverse health effect	A function of the probability of an adverse health effect and the severity of that effect consequential to a hazard(s) in food	CAC (1996)

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# HYGRAM<sup>®</sup> AS A DECISION SUPPORT TOOL

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People involved in the food sector and especially in food safety management, either coming from the food industry or competent authorities, are often asked to decide on issues concerning food safety. One can easily understand the importance of such decisions that may have a direct or indirect effect on consumer's health. These decisions are not always easy to make since a lot of factors might influence a certain scenario or certain case. Moreover the legislative framework covering the food sector only provides general directions whereas the reality is far more complicated. It is important to have a clear concept of the objectives that need to be met and assess the risks that are posed according to their importance and influence upon the product's safety level separately in each case. In this direction and due to the necessity for decision making, HYGRAM<sup>®</sup> (<http://hygram.vtt.fi>), which is a computer – aided model, was developed to facilitate the risk analysis process in food safety management.

HYGRAM<sup>®</sup> -2.0 is a semiquantitative model for assessment of most important hygienic hazards in companies. This practical tool has been developed in cooperation between the Finnish Food Safety Authority EVIRA and the Technical Research Centre VTT and has been finalized in 2007. The model construction includes parts of microbiological risk assessment as modules, and it familiarizes the user with the basics of risk assessment. The program consists of the following modules: Background module, 14 Hygiene modules and 14 Hazard modules, HACCP-module, Databank and Results module. Hazard modules contain modules for 11 pathogenic bacteria, moulds, viruses (norovirus) and a module for assessing physical and chemical risks. The users also have the option of creating their own hazard modules. The modules where hygienic practices, microbiological or other hazards are assessed, mainly account for the exposure assessment part of risk assessment. Risk estimates are presented as tables and illustrative figures. In HACCP-module it is possible to build and evaluate a HACCP-system.



First the user has to fill in all the background information by describing the process flow and product(s). In this step is important that the user identifies possible risks that relate to each one of the process steps. The user has then to select either a hygiene or hazard module from the list. The next step is to evaluate the probability and severity of a possible non-conformity or contamination for each one of the process steps which affects the product's safety and originates from the module under evaluation. Each module should be evaluated separately although sometimes it is possible to identify minor overlapping between different modules.

The certain computer aided model can be a useful tool for both food business operators and competent authorities. HYGRAM<sup>®</sup> is freely available for own use on the internet (<http://hygram.vtt.fi>) and its launching is not difficult since there are instructions of how to use it available on the front-page of the program, both as Quick user guide and User manual. The databank, also available within the model, is a good source of information concerning the most frequently food-related micro-organisms, of topics regarding the implementation of HACCP systems and general information about risk analysis.

## **FOOD BUSINESS OPERATORS**

The HYGRAM<sup>®</sup> model is a very efficient tool for the food producing companies. Companies can use it in order to apply some of the basic concepts of risk assessment on their products and processes and identify those steps where the greatest risks are posed in order to make the necessary improvements. Generally, it is well known that the industry is legally responsible for producing safe foods. At the basis of safe food production the aim is the design and manufacture of products with a good safety record. Informed and qualified judgement based on potential microbiological and other hazards and necessary control measures within the HACCP concept are used when new products are developed. The HYGRAM<sup>®</sup> model can be a very useful tool when designing new products, utilizing new production processes or changing manufacturing specifications. Both scientific knowledge and practical experience is needed to use the HYGRAM<sup>®</sup> model efficiently. The model combines both qualitative and quantitative elements and the assessor conducts the exposure assessment semi-quantitatively by scaling both the probability and the severity of the risks.

The goal of HYGRAM<sup>®</sup> is to provide a practical and easy-to-use model which can be used in conducting hazard analysis, in choosing effective critical control points with critical limits and in quantifying risks in such way that the different processes can be compared. Towards that prospective the HYGRAM<sup>®</sup> model has proven to be a useful and efficient tool that manages to combine semi-quantitative hazard analysis tailored to the users' needs. It should be stressed that hazard analysis is not a qualitative but a quantitative exercise. The ICMSF expressed as early as 1988, that the analysis of hazards must be quantitative, if it is to be meaningful. This point is raised because there is a persistent misconception that the HACCP system is qualitative and that risk assessment is quantitative. The concept of an 'acceptable level of a hazard' is as fundamental to the HACCP concept as 'acceptable level of risk' is to the risk assessment concept. However it should be realized that the two concepts are different and need to be kept as separate systems because they serve different purposes.

Through HACCP and the use of the HYGRAM<sup>®</sup> model industrial safety assessment assures the production of safe food products focusing either on a single product, production site/line or the whole process. The evaluation of a single product can be performed quite easily whereas when evaluating a process line or the whole process the person(s) conducting the assessment should bear in mind all the relevant parameters that affect the commodities produced under similar conditions.

Moreover, the users of HYGRAM<sup>®</sup> must have competence in food hygiene. It is essential to calibrate evaluations when comparing processes to each other. In brief the use of the HYGRAM<sup>®</sup> model within food industries as a decision tool can support not the only hazard identification and characterisation, but also the determination of critical control points and the assessment of their functionality and effect /allocation. The prioritization of hazards can lead to implementation of better control measures and stricter critical limits where necessary. This is why HYGRAM<sup>®</sup> differentiates from the so called traditional hazard analysis since it can be used as a risk assessment tool forming a link between hazard and risk analysis. Besides its use as a decision support tool its role as an orientation tool for new employees should not be underestimated. Furthermore, it can be used as a documentation tool, as an internal auditing tool, as an electronic record keeping database of the HACCP or Own checking System and can also be a very good way of collecting information needed for risk assessment.

Therefore HYGRAM<sup>®</sup> model can be considered as an operative tool for risk quantification. With HYGRAM<sup>®</sup> the risk quantification can be carried out in a practical and transparent way, because the results point directly to the process steps and the hygienic factors. The declarations are also listed into the memorandum that HYGRAM<sup>®</sup> includes and can be used every time a re-evaluation takes place. HYGRAM<sup>®</sup> model can also be used as an educational tool for new employees in food enterprises in order for them to get more familiar with the concepts of hazard and risk and also the principles of HACCP and any other Own checking Systems that might be in use by the company. Generally it can be considered as a good method for stratifying hazards and evaluating them in a certain way and at the same time guiding all the assessors to perform their assessments in a comparable way. One should keep in mind that the assessments carried out using the HYGRAM<sup>®</sup> model need to be done by different users in order to create a complete picture and each assessor should note his/her comments so that they are available for others.

## **COMPETENT AUTHORITIES**

Inspectors belonging either to competent authorities or other inspection bodies are often asked to make or support decisions or even evaluate a certain case in the area of food safety. Furthermore, they are often asked to decide upon the frequency of inspection in a certain food industry according to the risks posed. Moreover, they need to re-evaluate cases when a new product is produced or a new production line is incorporated to an already existing one, when production procedure changes (new raw materials, new equipment, and new products) or even when consumer patterns and habits change.

Profiling of a company by using the HYGRAM<sup>®</sup> model allows inspectors to provide their expert judgment and decide upon issues concerning food safety in a short period of time. HYGRAM<sup>®</sup> model allows them to define through a transparent procedure for the frequency of inspections and follow ups in an industry based on the principles of risk assessment. In this way the results are expressed in a uniform way. Moreover HYGRAM<sup>®</sup> can also be used as an auditing and educational tool by competent authorities. The structure of HYGRAM<sup>®</sup> model allows the inspectors to assess the CCPs in HACCP or Own checking System and justify their decision when changes need to be made or corrective actions need to be implemented within an industry.

## SUMMARY

HYGRAM<sup>®</sup> offers a practical way of assessing and tracking risks on company level. The HACCP group is guided by HYGRAM<sup>®</sup> to conduct the whole procedure systematically. Food companies will find the databank, with information on the hazards, the guidelines of HACCP and risk analysis, to be an important and useful tool in obtaining updated knowledge, making assessments and taking decisions. In addition, auditors, food inspectors, and the quality assurance personnel may use the guiding hygiene and hazard module texts as a checklist and a documentation record. HYGRAM<sup>®</sup> can also be considered as a useful education method for teaching own checking and hygiene.

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# PROBE-SPECIFIC DETECTION OF *SALMONELLA* IN CHICKEN MEAT BY LIGHTCYCLER PCR

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**Aim:** The aim of this study is to detect *Salmonella* from chicken meat by probe-specific real-time PCR (RT-PCR). **Methods and results:** Frozen laboratory culture *Salmonella* Enteritidis strain 222 was resuscitated on buffered peptone water and checked for purity by streaking on to 5% sheep blood agar and XLT4 agars. Ten fold dilutions up to  $10^{-8}$  was performed from  $4.0 \times 10^9$  cfu ml<sup>-1</sup> count stock culture, and 1 ml from each dilution was used in DNA isolation by Highpure Foodproof I kit (Roche). Template DNAs from all dilutions were utilized in RT-PCR as indicated by LightCycler® Foodproof *Salmonella* Detection Kit (Roche). *Salmonella* detection sensitivity of our system was determined as  $4.0 \times 10^2$  cfu ml<sup>-1</sup> *Salmonella* cell DNA. Further, this system was used to detect the presence of *Salmonella* in chicken meat samples. For this, DNAs obtained as above from 24 h lactose broth and 18 h buffered peptone water pre-enrichment cultures of 46 chicken meat samples were used as template in probe-specific real-time PCR. Results revealed that 23 (50.0%) and 32 (69.6%) of the lactose broth and buffered peptone water (BPW) pre-enrichments harboured *Salmonella*. **Conclusions:** The LightCycler probe-specific RT-PCR system detects *Salmonella* in pre-enriched cultures of chicken meat samples, and can be used as a rapid detection method, complementary to gold standard FDA-BAM and ISO 6579-2002 bacteriology methods. Also pre-enrichment using BPW yielded significantly higher *Salmonella* percentage, which indicates the use of BPW is appropriate in template preparation for this system.

# **PRACTICAL EXAMPLE OF PROCESS HYGIENE IN ESTONIAN DAIRIES**

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In the frame of SAFOODNET project FP6-022808-206 (food safety and hygiene networking within new member states and associated candidate countries) the second pilot case was performed during summer 2007 in Estonia. The aim of this study was to evaluate cleanliness of dairy plants selected for the survey and to carry out some innovations for improving process hygiene at the places. based on the questionnaires answered beforehand by the hygiene managers for different dairy companies sampling places have been selected.

In the experimental part of the study hygiene level was evaluated before and after improvement of the hygiene practices in all the four Estonian dairies. sampling procedures have been carried out by our students Liina Kutsar and Marite Mets and their supervisor Assistant Professor Tiina Veskus under the supervision and direct participation of our Finnish colleagues from the SAFOODNET project Gun Wirtanen, Satu Salo and Marjaana Rätto.

Hygiene survey results demonstrated that hygiene level between two samplings did not improve significantly process hygiene after implementation of some cleaning innovations. But, cleaning methods used in Estonian dairies were effective to provide the required hygiene level of direct contact surfaces. Cleanliness of indirect product contact surfaces and environmental samples were containing higher microbial counts. Protective clothing, handwashing sinks and drains were potential sources of contamination. Microbial quality of water used in production was generally in acceptable limits.

Results of detecting pathogens demonstrated that cleaning methods used in Estonian dairies should be more effective to minimize the potential risk of pathogens. In the report all the data will be given in details.

# STATE-OF-ART IN HYGIENIC DESIGN IN FOOD INDUSTRY

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The machinery directive which is the basic European law text has been enforced since the 1<sup>st</sup> of January 1995. The directive only addresses hygienic design in very general terms and the specific information is to be expressed through a set of standards of which Food Machinery – Basic requirements EN 1672-2 is the basic document, which addresses some of the basic explanations of proper and improper design but this document needs to be followed by some specific standards covering actual processes. As of 18<sup>th</sup> September 2008 there are 40 such specific standards within the framework of food machinery being approved. This does not even come close to covering the needed range of processing equipment used in the food industry.

An other side of the EU regulation is the Framework Regulation (EC) 1831/2003 (L338/4) which states that food contact materials shall be safe, that they shall not transfer their components into the food in quantities that could endanger human health or change the composition of the food in an unacceptable way deteriorate the taste and odour of foodstuffs. The regulation states that materials under normal use or under predictable conditions must not release parts or components to food products in amounts which could be dangerous for the health of people or cause unacceptable changes to the composition of the food product i.e. change the colour, smell, taste or any other physical characteristics of the food product. Also the food producers should be able to trace the materials used.

Even before the EU regulations were instated the private organisation European Hygienic Engineering and Design Group (EHEDG) [www.ehedg.org](http://www.ehedg.org) was established. EHEDG provided state of the art information back in the 90's and is still doing so. The lack of a full range of specific standards makes EHEDG's 35 guidelines and test methods highly needed for the conscious food producer and equipment manufacturer. It should also be realised that even if all standards were made the

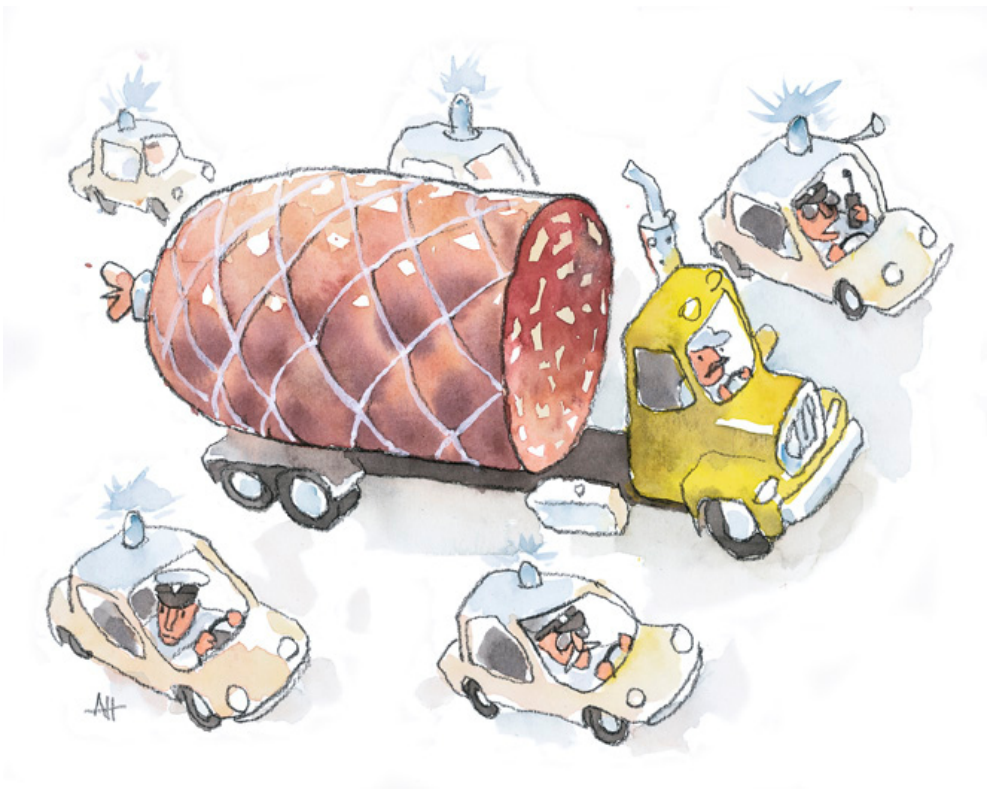
EHEDG documents would still very useful. EHEDG has a basic set of guidelines being numbers 8 Hygienic equipment design criteria, 10 Hygienic design of closed equipment for the processing of liquid food, 13 Hygienic design of equipment for open processing and 22 General hygienic design criteria for the safe processing of dry particulate materials. These guidelines can be acquired at a reduced price as a set and constitutes a good introduction to hygienic design of food processing equipment. There is also no doubt that EHEDG continues to be a dominating player with a continuous introduction of new guidelines especially number 34 concerning Integration of hygienic and aseptic systems which is a horizontal guideline being a frame bringing many of the other guidelines together.

Concerning the US market it may be useful to refer to the 3-A Sanitary Standards, Inc. [www.3-A.org](http://www.3-A.org) which has produced more than 70 sanitary standards and another 12 documents on “Accepted practices”. Also the National Sanitation Foundation (NSF) [www.nsf.org](http://www.nsf.org) has produced documents like NSF 14159-99 Safety of Machinery – Hygiene requirements for the design of machinery and furthermore more than 50 specific standards on the issues of health & safety.

On the international scene the International Organisation for Standardisation (ISO) has the standard ISO 14159-2002 Safety of Machinery – Hygiene requirements for the design of machinery which in many cases are similar to EN 1672-2 but not as strict in some cases. In recent years also the costumers especially the retailers has made guidelines for their suppliers to follow. In the UK BRC – The British Retail Consortium is a trade organisation of large supermarket chains in UK sets common demands for their producers. See more at [www.BRC.org.uk](http://www.BRC.org.uk). In mainland Europe the IFS – was established in 2002 of de German retailers from HDE (Hauptverband des Deutschen Einzelhandels) and in 2003 did the French food retailers from FCD (Fédération des entreprises du Commerce et de la Distribution) enter the IFS working party and took part in the last revision – version 4.



## PARTICIPANT ABSTRACTS ON RISK ASSESSMENT



# ELABORATION OF A CONCEPTUAL MODEL FOR PREVENTING MOULD CONTAMINATION DURING PRODUCTION IN BAKERIES

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The **aim** of the work is the elaboration of a conceptual model for prevention the contamination with moulds in bakery industry. A key point was the organisation of production, following the technological phases, starting from the raw material – wheat (*Triticum aestivum*) until the flour production; which is the raw material for bread and pasta. **Materiaial & Methods:** The samples were gathered from Baneasa Mill, following the content of moulds (according to standard SR ISO 7954:2001 – Microbiology. General directions for the count of moulds. The methods at 25<sup>0</sup>C) and deoxynivalenol – DON (using ELISA method). The technological phases were: gross wheat, clear wheat, products from the main passage of milling and flour / semolina / husk as final products. The initial data showed a low content of moulds and DON in the products (flour, semolina, bread), while at the refusals the content is higher (above 750 µg/kg). **Conclusion:** In order to reduce the content of moulds we propose to find out a correlation between the humidity (temperature) of products, the amount of water added in the process and the growth of moulds. We know also that the heat evolved during the milling process (at the platens) is important for the growth of the moulds, and for the future we should follow this aspect. We sustain also the using of rapid tests in the reception process of wheat. Their work represents the partial conclusion of the project “An incorporated system for reduction of contamination with moulds in bread industry in order to increase the food safety” financed by Ministry of Education, Research and Youth.

# PREVENTION SYSTEM DEVELOPMENT FOR TOXIGENIC FUNGI IN AGROFOOD PRODUCTS BY PREDICTIVE MICROBIOLOGY AND MOLECULAR BIOLOGY TECHNIQUES

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The **aim** of this project was to determinate the level of DON mycotoxin produced by *Fusarium graminearum* 37 depending of the time and temperature of inoculation. Trichotecenes (from which deoxinivalenol mycotoxin takes part) represents a group of mycotoxins with similar chemical structures that causes serious illness to both humans and animals manifested in one or more morpho-clinical symptoms: diarrhea, brain storm, skin necrosis, miscarriage, hemorrhage and necrosis in organs and tissues, degeneration of bone marrow. Trichotecenes are not stable in cereal and fodder during long periods. It was not detected in final products stored at 4°C 3–6 months. **Material & Methods:** The quantification of DON mycotoxin was realized using culture media inoculated with *F. graminearum* 37. The inoculum was obtained using following steps: the strain kept on Czapek Dox agar was incubated in Czapek Dox broth. The inoculum was incubated at 8 different temperatures: 12°C, 16°C, 20°C, 23°C, 26°C, 30°C, 33°C, 36°C. For each temperature of incubation, 2-ml samples have been taken once every second day obtaining nine samples for every temperature. After that, the samples were kept in Eppendorf tubes, in the fridge (4°C) until mycotoxin analysis. Detection of DON mycotoxin was performed using a ELISA method. The RIDASCREEN® DON KIT quantifies at ppb-levels (detection limit 18.5 ppb). All 72 samples were centrifuged at 15300 rpm, for 15 min at 4°C. There were more repetition carried out for testing of toxicogen potential of DON mycotoxin production and thus a mean of all results were given. **Results:** The biggest concentration (1385 ppb) of DON mycotoxin for the *F. graminearum* 37 strain was obtained at incubation temperature of 26°C, at the 17<sup>th</sup> day. At this temperature (26°C) an increase in the DON mycotoxin concentration was seen along the 17 days of incubation. The start level of DON mycotoxin at the first day was 89.44 ppb.

# OVERVIEW OF THE OFFICIAL CONTROL RESULTS OF FEED FOR THE IDENTIFICATION OF RISKS

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In the recent years, the introduction of the food chain approach, which recognizes that responsibility for the supply of safe food, has served to highlight the importance of safety feed which is on its very beginning. Development of improved practises in the feed production and development of sampling and analytical techniques are necessary for achieving proper food safety standards. In the same time, development work on the application of the risk analysis framework has facilitated understanding of the potential impact of animal feed safety on public health. Therefore, consumers are increasingly aware of food safety problems which are linked with feed production. As animal feed is an important route by which hazards can be enter in the human chain, its safety must be properly assessed. Those hazards can be biological, chemical and physical. Each of them is associated with particular sources and routes of contamination and exposure. The list of potential hazards is very large and constantly evolving. A multidisciplinary approach to risk assessment therefore is needed. In order to assess the risk, the first step that has to be done is risk identification, which is dealing with identification of any undesirable substances and micro-organisms which may be naturally present in feed or introduced during the production, distribution or transport. The main purpose of this paper is to present the results of official control of feed, point out on main problems and shortages and give a few suggestions how to minimise them.

# FOOD SAFETY SYSTEMS IN REPUBLIC OF CROATIA

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Globalization of trade and food production enlarges the risk of spreading infections as well as problems that arose from food borne diseases. Due to this reasons, food borne pathogens can be spread miles away from place of food production or packaging, even to other continents. For reducing risk of food borne illnesses, this fact is crucial for understanding the ways of entering and spreading the pathogens in food chain. In addition, this emphasizes the need for risk assessment of pathogens because of damages that they can cause on international level, as well as on national. By establishing new standards and regulation in the field of food safety in late 20 century, requests become considerably more rigid. Those requests refer especially on international trade, but they are implemented in national frames as well. However, measures that are implemented in food safety framework differ from country to country, resulting in trade dissidences. Standards, guidelines and recommendations adopted from Codex Alimentarius Commission (CAC) as well as international food trade agreements like the one from World Trade Organization (WTO), have significant role in entire system by protecting consumer's health and guarantying fair trade. Due to approaching to European Community, Croatia intensively work on food safety issues following the new approach based on risk analysis and "from farm to fork" concept and by implementing prerequisite programs for safe and sound food products.

# AIRBORNE FUNGI IN A TURKISH OLIVE PROCESSING PLANT

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Studies employing gravity settling culture plates (GSC) were conducted in order to analyse the airborne fungi of an olive processing (ripe pitted, natural calamata pitted and whole olive) plant in Izmir, Turkey, in May 2008. Sampling procedure for airborne fungi was performed 8 times during the processing. Numbers and types of airborne fungi in the air of olive processing areas were investigated by exposing a Petri dish of malt extract and potato dextrose agar medium for 15 min and then counting the number of colonies which develop after incubation at 25°C for 7 d.. Moulds were identified according to Pitt and Hocking (1997) and Samson *et al.* (1996). All the strains were tested for morphological characters. Results revealed a variety of fungal spores belonging to different genera. As a result, 63 mould species belonging to 5 genera were isolated by means of pure culture methods. Environmental assessment of fungal spores by GSC method revealed that the most frequent genera of fungi were *Cladosporium*, *Alternaria*, *Penicillium*, *Aspergillus*, *Rhizopus* and some unidentified fungi. In the microflora of the airborne fungal samples investigated a coexistence of lactic acid bacteria, *Bacillus*, and yeasts was observed, but in all samples the presence of moulds was dominant. Sources of contamination at olive processing plants include personnel activities, ventilation systems, floor drains and water applied under pressure during cleaning and sanitizing procedures. These microbes could contaminate the product via contact with processing surfaces, where they may adhere and form biofilms. Air is only one of the sources that may come into contact with the product during many stages of handling, processing, storage and packaging. GSC is most frequently used for evaluating the microbiological quality of air in plants. According to the American Public Health Association standard the maximum contamination rate from air is 30 CFU·cm<sup>-2</sup>·week<sup>-1</sup> in food processing areas, when evaluated by GSC. In this study the mean log<sub>10</sub> count of mould per plate (duplicated) was between

1.88 and 4.78. Microbiological air quality was not acceptable in the olive processing plant because the concentrations of moulds were higher than 30 CFU·cm<sup>-2</sup> and the predominant genus identified was *Penicillium* (75%). The highest mean counts (> 300 CFU/plate) of airborne fungi were detected in the olive receiving, filling and pitting areas, while the fermentation in tanks, final product filling and storage area showed the lowest mean counts (76–150 CFU/plate) of airborne fungi. The information about the distribution of the biological contaminants in SME food processing plant areas is limited. There is also lack of the techniques of quantification in order to assess exposure and risk characterization. Therefore assessing the impact of airborne fungi is important in respect of toxin producing moulds and their consumer risk. The results of this study demonstrate that the microbiological quality control of air in SME olive processing plants could be improved and optimized using chemical sanitizers and application of good manufacturing practices.

# MICROBIOLOGICAL RISK ASSESSMENT IN PUBLIC CATERING ESTABLISHMENTS

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Microbial contamination of both thermally processed and thermally unprocessed ready-to-eat foods was studied with the help of methods of microbial analysis. For the first time in Latvia methods of mathematical statistics have been used to accomplish quantitative microbiological risk assessment and to ensure science-based risk management and risk communication proposals for both control of distribution of microbiological contamination and prevention of outbreaks of group enteric infectious diseases in public catering establishments:

- risk of microbial contamination of ready-to-eat foods has been estimated based on methods of both food technological processing and food composition;
- risk of microbial contamination of surface of environmental objects has been assessed that results from efficiency of cleaning and disinfections procedures;
- results of introduction of good hygiene practice in different public catering establishments have been analysed;
- similar trends in distribution of microbial contamination have been discovered within group infectious disease outbreaks and state's monitoring and control;
- poor efficiency of self-control procedures in public catering establishments has been assessed;
- results of microbial risk assessment have been applied for purposeful control of microbiological contamination with help of Petrifilm rapid test method.

Statistically significant differences in microbiological contamination have been established that result from hygiene practice in public catering establishments. Methodology of risk analysis has been recommended for analysis of trends in transmission of microbiological contamination, purposeful implementation of procedures for control of contamination, and for timely prevention of foodborne enteric infectious disease outbreaks in public catering establishments to promote public health protection.



# MONITORING OF MICROBES ON PROCESS SURFACES AND PERSONNEL

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**Introduction:** The complexity of food manufacturing plants continues to increase and the potential for issues adversely affecting the consumer increases accordingly. GMP policies, HACCP programs, plant policies and practices, production parameters, preventive maintenance and sanitation/hygiene programs, along with that the facilities should meet their obligations. Plant inspections are used by several different entities to achieve the same goal. Regulatory agencies utilize plant inspections for the enforcement of food laws.

A company's customers utilize inspections to determine the risks of doing business with a particular firm, using either their own resources or a third-party professional organization, to conduct the inspection. Perhaps the most important aspect of the inspection program, however, is the self-inspection program undertaken by the own personnel monitoring the conditions in the plant. The personnel must identify potential food safety risks and take actions to correct deficiencies developed. In this study, the way in which the food industry conforms to this legislation was analyzed through a case study of Turkey province, which generally has all the structural characteristics of most sector e.g. dairy, oil, catering, meat, beverage etc. in Turkey. The current work was performed during 2007–2008 as a part of the big supermarket chain inspection project.

The **objective** of this study; was to determine the hygiene of the food industry through monitoring the adherence of total aerobic bacteria; *Escherichia coli* and *Staphylococcus aureus* on surfaces and personnel hygiene. For this purpose; samples were collected from 290 food companies.

**Method:** Samples were taken with contact agars (Chromogenic Total viable count, Chromogenic *E. coli* & Chromogenic *S. aureus*). All the analysis were performed using standard cultivation techniques.

**Results:** Within all surface samples the highest levels of microbiological level were found at frozen food sector (75%), meat sector (66.7%), catering sector (63.6%); respectively. The survey results indicated that spices sector and marmalade and jam sector are concentrating more on the hygiene of food surfaces. When comparing personnel hygiene samples; the milk and milk products and catering sectors have the highest levels of *E. coli* within all sectors. The survey also showed that several surface areas are problematic. Improved hygiene in all food sector can be gained through proper cleaning and proper monitoring of the surface hygiene.

# MICROBIAL RISKS ASSOCIATED WITH TRADITIONAL CHEESES

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Nowadays, there is a growing interest towards traditional cheeses. These cheeses are especially popular in Mediterranean countries due to their unique flavor and texture. Traditionally they are produced from raw milk of cow, sheep or goat and fermented by naturally occurring indigenous lactic acid bacteria. The quality of these cheeses is based on the microbial associations within the respective region and production recipes change from one town to another and even from one manufacturer to another within the same town. Hygiene practices in the dairy and in the cheese rooms are the most important criterion in traditional cheese manufacturing. Based on the identification of hazards, microorganisms of concern are *Escherichia coli* O157:H7, *Salmonella*, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Staphylococcus aureus* (toxin producing strain), may contaminate milk and grow in traditional cheeses when raw milk is utilized during manufacturing. Good quality of raw milk from healthy animal contains fewer pathogenic microorganisms and also contain proteins (lactoferrin) and enzymes (lactoperoxidase) that inhibit or eliminate these microorganisms. During cheese manufacturing, conditions need to be favourable for the growth of lactic acid bacteria and the elimination of pathogenic microorganisms by controlling the variables such as temperature, moisture, and acidity. In one study conducted in UK, the quality of raw milk cheeses were found to be unsatisfactory due to the levels of *S. aureus* at  $10^4$  cfu/g, *E. coli* at  $10^5$  cfu/g, and *L. monocytogenes* at  $10^2$  cfu/g. Cheeses having unsatisfactory quality are frequently sampled from dairies having little or no confidence in management and control systems, and stored at inappropriate temperatures ( $> 8^\circ\text{C}$ ). Some precautions need to be taken regarding raw milk traditional cheeses. These should not be sold unripened for consumption. Traditional cheese manufacturers themselves play a significant role in monitoring the safety of their products and implement and maintain a permanent procedure based on the hazard analysis and critical control point (HACCP) principles.

# FOOD SAFETY IN PRODUCTION AND PROCESSING OF BLUEBERRY

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Food safety is one of the most important concepts related to human healthy and illness. The aim se of this project was to assure food safety on the food chain of the blueberries production and processing. The steps in the study were: identification of sources of contamination, potential hazard during blueberries breeding; establishment of suitable control measures for the prevention, elimination and/or the reduction of the important potential hazards during blueberries obtaining, identification of the potential hazards (microbiological, chemical and physical) during on blueberries processing, evaluation of the potential risks depending on the gravity and probability of happening. The food safety management system in obtaining reliable foods using Hazard Analysis and Critical Control Point (HACCP) was analysed. A HACCP-based system was use as the key instrument in ensuring the quality along food chain. The study consisted of identification, evaluation and management of all physical, chemical and biological hazards which can occur within production, processing, distribution and retail chain of blueberry products. The risk analyse is specific for each technological process in every company. The 'Decisional Tree' -scheme was used for establishing the critical control points in the blueberries chain in accordance with *Codex Alimentarius*. The 'Decisional Tree' was used for each process step and also for each identified hazard within the chain on canning blueberries to establish the critical control points (CCPs). One CCP in the pasteurization was found based on analysing the process.

# THE DEVELOPMENT OF A HACCP AND QUALITY MANAGEMENT SYSTEM IN A FOOD MANUFACTURING COMPANY

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A number of food manufacturing companies are interested in developing their quality management system. Several public and private standards on food safety and quality, including ISO 9001, ISO 22000 and BRC global food standard, are in use. Well-prepared and working quality management system is not only beneficial to the company, it also simplifies the work of the specialists in the companies. If creating the quality management system is elective, then establishing the own checking system (OCP) based on the critical control point (CCP) is compulsory in all European Union countries. According to the regulation No. 852/2004 of the European Parliament and of the Council of 29 April 2004, on the hygiene of foodstuffs, food manufacturing industries should establish and operate food safety programmes and procedures based the hazard analysis and critical control point (HACCP) principles as the HACCP system is an instrument to help food business operators attain a higher standard of food safety (Doménech *et al.*, 2007). Most of the quality management standards assume that before developing the standard, the HACCP system is properly in use. It means that HACCP is an important part of the standard. Implementing any of the quality standards needs a training of the personnel and devotion of the top managers. It is very important that all employees, starting from the production workers and finishing with the top managers understand the meaning and the importance of HACCP and the need to develop the quality management system. Introducing HACCP to small bakeries is particularly difficult as many products are produced and staff familiar with HACCP are lacking. Before making an HACCP concept, hygienic weak links must be removed by ensuring Good Manufacturing Practice. Dirty equipment and lack of cooking are the hygienic weak points in these products (Leitenberger & Röcker, 1998). The team of the quality management system should consist of the specialist from a different fields – production manager, quality manager, technologists, logistics and

warehouse specialists, maintenance specialists etc, because the creation of the system needs a real teamwork. There is of course also a negative side. Currently, there is proliferation of standards worldwide. One effect is that, in particular, companies from developing countries and emerging economies have problems to comply with these standards. Another important effect is increasing marginal costs of certification and accreditation, which also puts pressure on company profits in industrialized countries (Trienekens *et al.*, 2008).

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# PREVALENCE AND GENETIC CHARACTERIZATION OF *LISTERIA MONOCYTOGENES* IN RETAIL BROILER MEAT IN ESTONIA

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**Aims:** To study the prevalence and genetic diversity of *Listeria monocytogenes* in raw broiler legs at the retail level in Estonia.

**Materials:** A total of 240 raw broiler legs (120 Estonian and 120 imported from Denmark, Finland, Hungary, Sweden and USA) from 12 retail stores in the two largest cities of Estonia – Tallinn and Tartu – were investigated in 2002.

**Methods:** *L. monocytogenes* was detected using the ISO method 11290-1:1996 and the identification was based on beta-hemolysis, Gram, catalase and API-Listeria tests. PFGE (pulsed-field gel electrophoresis) typing with *AscI* restriction enzyme was used on 169 *L. monocytogenes* isolates and 1–3 strains from each PFGE type were further selected for serotyping.

**Results:** Of the raw broiler legs, 70% were positive for *L. monocytogenes*. The prevalence of *L. monocytogenes* in broiler legs of Estonian origin (88%) was significantly higher than in broiler legs of foreign origin (53%) ( $P < 0.001$ ). Altogether, 169 (106 Estonian, 63 imported) isolates were characterized by PFGE-typing using restriction enzyme *AscI*. The isolates showed a wide genetic diversity, 35 different PFGE types being obtained. Of these, 11 PFGE types were only isolated from Estonian broiler legs, 4 from Danish, 2 from Finnish and 4 from Hungarian. Isolates from various countries represented 14 PFGE types. The strains sharing the same PFGE types were recovered in broiler legs of Estonian origin from different stores over several months. *L. monocytogenes* was serotyped in 71 isolates including all PFGE types and the serotypes 1/2a, 1/2b and 4b were obtained. Serotype 1/2a accounted for 96% of the isolates.

**Conclusions:** The overall prevalence of *L. monocytogenes* in raw poultry meat obtained from retail outlets in Estonia was high. The number of *L. monocytogenes*-positive samples in poultry meat of Estonian origin was higher than in imported ones. The genetic diversity of *L. monocytogenes* was high. Same *L. monocytogenes* PFGE genotypes were identified among Estonian and imported isolates. Serotype 1/2a was predominant in Estonian poultry products. The further investigations at abattoirs and processing plants level are needed to prevent the continuous contamination.



# POTENTIAL THREAT OF TYRAMINE PRODUCTION IN ENTEROCOCCI ISOLATED FROM DAIRY PRODUCTS

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Biogenic amines (BA) found in a number of foods are organic bases with an aliphatic, aromatic, or heterocyclic structures. They are mainly produced by the microbial decarboxylation of amino acids. Biogenic amine formation may occur in various dairy products such as cheeses, fermented foods and beverages due to amino acid decarboxylase activity of enterococci. Biogenic amines and polyamines occur in various tissues and play an important role in cell regeneration and differentiation. But, in many cases if BA intake is more than the normal value or if the individual's natural detoxification mechanisms are inhibited or deficient than even a low BA concentration can be problematic. Tyramine and histamine are the most studied amino acids because they are able to evoke symptoms e.g. alterations in blood pressure, head ache, urticaria, nausea and vomiting. Hence, presence of biogenic amines in the food stuffs may result to food poisoning and could imply food exportation problems. The **aim** of the study was to determine the capacity of different strains of enterococci from various dairy products to produce tyramine. **Methods:** In total 75 different strains of enterococci from various dairy products were studied. A multiplex PCR was designed for the genotypic differentiation of various *Enterococcus* strains and to determine the presence to tyramine producing (*tyrdc*) gene. **Results:** *E. faecalis* followed by *E. faecium* was found to be the most prominent strains present in dairy products. Presence of *E. mundtii*, *E. malodoratus*, *E. durans*, *E. casseliflavus*, *E. raffinosus* was also found but to a lesser extent. 80% of all strains were found to be carrying *tyrdc* gene responsible for tyramine production. *E. faecalis* was most active and *E. casseliflavus* least active in producing tyramine. **Conclusions:** In the dairy products and raw milk the prevailing strain was *E. faecalis* followed by *E. faecium* and than other strains. This might prove to be a potential threat for the society because of their capacity to produce tyramine. Hence, it becomes necessary to monitor various dairy and related products for the presence of tyramine at the industrial and farm level.

# ASSESSMENT OF PRIMARY SCHOOL CANTEEN IN HACCP APPROACH

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**Aim:** This research was planned and carried out in order to examine service quality, physical conditions, personnel, food and beverage quality of the canteens which serve students of the primary schools in Province of Çankaya. **Methods:** This study carried out by questioner to and observations in primary school canteens. **Results:** It was determined that physical conditions of the canteens were not adequate, and the personnel of these canteens did not perfectly care about the hygiene neither of personnel nor in the environment. The hygienic quality of food was not always perfect. It was seen that only 17.5% of employee used cap and bonnet properly and 55% of them were not able to wash their hands properly. None of the school canteens used probe thermometers for measuring the internal temperature in potential hazardous foods properly in the intuitional food services' (purchasing, delivery, storage etc.) critical control points. Only 75% of the personnel is examined properly for necessary health tests within appropriate intervals. There is no possibility for having a bath (necessary for personnel hygiene) before and after working hours in the facilities belonging to the school canteens. It was also determined that the personnel did not have enough information about the importance of service quality of hygiene in intuitional food service establishments, of personnel, and of food. **Conclusion:** The personnel in the canteens have to be educated very well and HACCP procedures must be applied at all school canteens.

# **RISK ASSESSMENT AND RISK MANAGEMENT IN THE FOOD INDUSTRY**

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Food safety assurance in the food industry is very important in the aspect of gaining the faith of the customer on product. The determination of the risks, the risk assessment and the risk management must therefore be carried out correctly for each step of food production to gain customer trust. In this study, the risk analysis and the risk management were described and the importance in food safety is emphasized. In addition, risk analysis with critical control points for the Turkish delight production i.e. locum line was carried out as an example to food industry.

# **IMPLEMENTATION OF HAZARD ANALYSIS AT CRITICAL CONTROL POINTS – THE HACCP- SYSTEM TO TEHINA HALVAH (TAHIN HELVA)**

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Tehina halvah is an important traditional sweet that described as 'sweet meat' in United States of America. This food is produced with sugar, water, citric acid and tartaric acid; if needed glyucose syrup can also be used. Different taste materials like cocoa, hazelnut, pistachio nut etc. can be added. The production of this food needs careful working under hygienic conditions, because production of tehina halvah is a batch process and thus human is an important factor of the process. Chemical, physical and microbiological hazards can appear at the different process steps. In preventing problems, Hazard Analysis at the Critical Control Points (HACCP) system can be implemented. With using this system food control and safety can be obtained. The flow diagram of production, risk analysis and critical control points are stated and the limit values of the critical control points are also given in this system. In this study, HACCP system was applied to Tehina halvah production and trough this system the hazards can easily be demonstrated. It also shows in which places the hygiene must be improved.

# TESTING A REAL-TIME PCR DETECTION METHOD FOR *SALMONELLA* SPP. IN FOOD AND OTHER ENVIRONMENTAL SAMPLES

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*Salmonellae* are frequently causing food poisoning in the world, despite many preventive measures taken to control these microorganisms. In the United States and other industrialized countries declared cases of salmonellosis vary around 70,000–100,000. However the true incidence of food-borne *Salmonella* infection is estimated to be much higher, in the order of 4–5 million cases a year. The presence of *Salmonella* species in ready-to-eat food is considered significant though the level of contamination can be low. Isolation of *Salmonella* is therefore carried out by enriching *Salmonella* present in a defined weight or volume of food sample (normally 25 g). For environmental samples such as swabs and non-woven cloths, the entire sample is usually examined. A pre-enrichment resuscitation stage is incorporated to allow the recovery of injured cells. There is a variety of methods for detection of *Salmonella* from food samples. The suitability of methods can vary depending on the type of food being examined. Conventional bacteriological methods are often time consuming and tedious. The necessity to have fast and reliable results especially for perishable foodstuffs with short shelf life has led to the development of new molecular methods. In this study a molecular method iQ-Check *Salmonella* II is described and used. IQ-Check *Salmonella* II is a simple and rapid qualitative test allowing the detection of specific DNA sequences unique to *Salmonella* spp.. *Salmonella* spp. specific DNA sequences are amplified using real-time polymerase chain reaction (PCR) and detected simultaneously by means of fluorescent probes. Results from pre-enriched samples are obtained within a few hours. IQ-Check *Salmonella* II is specific for the *Salmonella* genus and the detection limit can be up to 1–10 CFU/25 g sample, according to the recommended enrichment.

**Method:** *Salmonella* were analyzed from different kind of samples such as surface samples taken with non woven cloths and cotton swabs, water filters, final products, raw materials and suspected colonies from contact plates using agar plates with Chromogenic *Salmonella* Medium (OXOID, Hampshire, UK), ISO methods for the isolation of *Salmonella* and IQ-Check *Salmonella* II. The surface samples and water filters (non-woven cloth, swab or filter) were placed into 5ml peptone saline, kept refrigerated for approximately 10 days and then transferred into pre-enrichment broth (buffered peptone water). The volume of enrichment broth used was 10 ml. All pre-enriched samples were incubated for 24h at 37°C and 1 ml was collected from each tube to be used for the PCR protocol. Final products and raw materials were analyzed according to the enrichment protocol recommended by the manufacturer of IQ-Check *Salmonella* II using 25 g of sample. Suspected colonies from contact plates with Chromogenic *Salmonella* Medium were collected using a loop, placed into test tubes containing 10 ml of buffered peptone water and incubated for 24h at 37°C. For both latter cases 1 ml of enriched sample was used for the PCR protocol. All samples were at the same time treated according to the *Salmonella* detection method in foods (NMKL method No 71 5.ed., 1999). The agars used for this purpose were Xylose Lysine Deoxychocolate and Brilliant Green Agar. Moreover suspected colonies grown on XLD and BGA were also tested serologically using the ANI™ *Salmonella* test (ANI BIOTECH OY, Helsinki, Finland).

**Results:** A total number of 248 samples were analyzed including both 11 positive and 10 negative controls (provided in the kit) and 6 positive and 1 negative samples (prepared from cultures of *Salmonella* spp.). One positive sample was identified. The results in Figure 1 shows that positive control gives the highest signal around 28 cycles whereas the positive sample appears around cycle 32. No other samples gave similar curves and the internal control (FAM) was valid (Figure 1). Serological ANI™ *Salmonella* test was performed for 16 samples using suspected *Salmonella* colonies from different solid media. The only positive reaction was from the same sample which was already identified to be *Salmonella*-positive with the real time-PCR method.

**Conclusions:** The real time-PCR method using the BIORAD iQ-Check™ *Salmonella* II Kit can be considered as a fast and reliable method for the detection of *Salmonella* spp. whereas other methods such as normal cultivation both on XLD and BGA agar and also on Chromogenic *Salmonella* Medium might lead to false

positive or negative results. The BIORAD iQ Check™ *Salmonella* detection method is efficient in detecting live *Salmonella* cells even in mixed populations and overcomes difficulties that may arise from the interpretation of results when using cultivation methods. The sensitivity and specificity of the method was found to be adequate according to the results confirmed with serological testing.

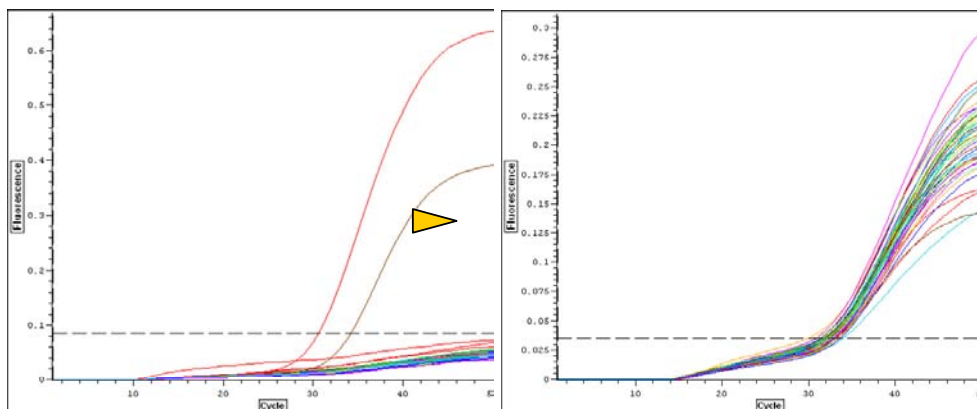


Figure 1. RT-PCR results on the left hand side and FAM fluorescence on the right hand side.

▶ Positive sample

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# **SALMONELLA PROFILE OF CHICKEN MEAT IN TURKEY: BACTERIOLOGY AND SEROLOGY FINDINGS**

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**Aim:** The aim of this study was to determine *Salmonella* profile in chicken meats by two standard internationally approved bacteriological methods, FDA-BAM and ISO6579-2002, and to obtain data on serotype information of the isolates.

**Methods and results:** A total of 46 chicken meat samples comprised of 29 wing, 2 neck, 10 thigh, and 5 whole chicken were collected from retail stores in Bursa, Turkey from October 2007 to June 2008, and were examined for the presence of *Salmonella* spp. by FDA-BAM and ISO6579-2002 methods. Forty two (91.3%) and 45 (97.8%) out of 46 samples were positive for *Salmonella* by FDA-BAM and ISO 6579-2002, respectively. After biochemical identification, 1–5 isolated colonies from selective xylose lysine desoxycholate and xylose lysine tergitol 4 agar plates were selected, subcultured on to Mac Conkey agar plates, and serotyping was performed by available antisera (*Salmonella* O antiserum Poly A containing somatic groups A, B, D, E1 (E2, E3), E4, L; *Salmonella* O antiserum Poly B containing somatic groups C1, C2, F, G, H; *Salmonella* O antiserum Factors 9; 12; 4; 5; Group E1 containing factors 3, 10; Group C1 containing factors 6, 7; and Group C2 containing factors 6, 8. Serotyping results revealed that 23 (50%) out of 46 samples harboured more than one *Salmonella* serotype.

**Conclusions:** These results indicated that *Salmonella* still is a problem in retail chicken meats of well-known brands in Turkey. ISO 6579-2002 complemented FDA-BAM method, and gave a more precise percentage on *Salmonella* presence on chicken meat samples. Detection of more than one isolate from a chicken meat sample occurred to be quite common, if the serotyping was applied by selecting multiple colonies of a sample plate. Further studies will be required for these isolates for full serotyping and genotyping, so that these strains can be related to salmonellosis cases and outbreaks in Turkey and worldwide.



# OCCURRENCE OF MRSA IN LIVE-STOCK FARMS

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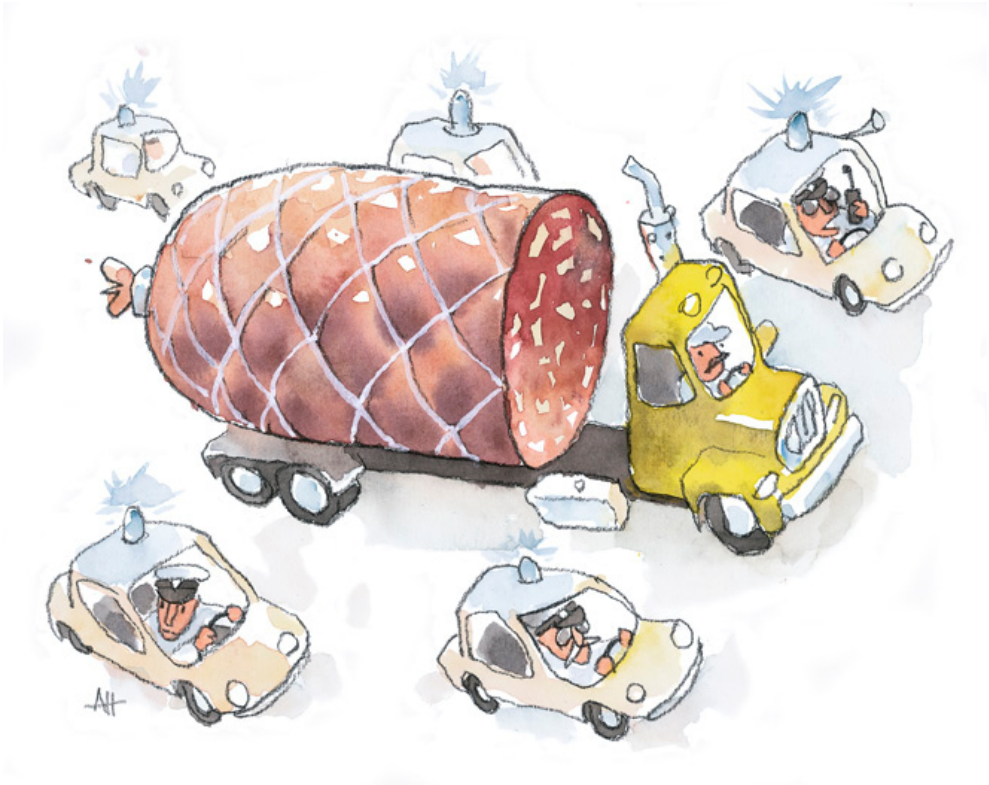
Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major human pathogen, having emerged first in hospitals and then expanding into a worldwide public health problem. The first description of methicillin resistant isolate of *Staphylococcus aureus* (MRSA), within the context of the hospitalization of patients, was in early sixtieth in the Great Britain. Since that the occurrence of MRSA has been monitored all around the world and in recent ten years the increasing trend in incidence of these strains has been noticed. MRSA are described in human medicine in connection with nosocomial infections above all. At the end of ninetieth the occurrence of MRSA was described in common population in USA and afterwards also in companion and food animals. The **aim** of our study was to find out the prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) in the live-stock farms in the Czech Republic.

**Methods & Material:** 445 samples, obtained from 13 live-stock farms, consisting of animal swabs and milk, swabs from environment and staff were examined. 10 MRSA isolates were found. 5 isolates were obtained from goat's milk on farm A. One isolate was obtained from cow's milk (farm B) and one from the swab of pig nose (farm C). 3 isolates were obtained from staff also on farm A (nasal swabs). All obtained MRSA isolates were genetically characterized by PCR and PFGE. By using PCR it was found out that almost all MRSA isolates (except two) encoded one or more staphylococcal enterotoxins. None of the isolates contained genes for exfoliative toxins, toxic-shock syndrome toxin-1 and Panton-Valentine leukocidin. According the results of PFGE all the MRSA isolates from farm A (of both animal and human origin) were the same pulsotype.

The **results** from this study revealed that the animals can be an important source of methicillin resistant staphylococci and represent potential danger for further spread to farmers, staff, their families, other animals and then to foodstuffs of animal origin.

As a **conclusion** of this study it can therefore be stated that it is important to monitor and then eliminate MRSA in the industry and prevent the spread of these strains.

## PARTICIPANT ABSTRACTS ON RISK MANAGEMENT



# **MICROBIOLOGICAL CLEANLINESS OF FOOD SERVICES IN NURSERIES, SCHOOLS AND SMALL FOOD PREMISES OF OSIJEK BARANYA COUNTY IN CORRELATION WITH THE NECESSITY OF HACCP SYSTEM IMPLEMENTATION**

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Microbiological cleanliness of food services is of the utmost importance for microbiological safety of food and prevention of foodborne diseases. In Croatia it is defined by Standards for microbiological cleanliness of food (OJ 46/1994, 125/2003 and 32/2004). This study was undertaken to determine the microbiological status of food contact surfaces and serving personnel hygiene in food services of nurseries, schools, catering and small food premises. Their actual microbiological status screening is of special interest because they are not strictly covered by Food Act to implement HACCP system. During one year period we examined 3035 samples from 221 services and premises using microbiological analyses according to HRN ISO 4833/2003 (aerobic mesophilic bacteria), HRN ISO 8523/1999 (enterobacteriaceae) and ISO 7899/2001 (*Streptococcus* D group) methods. The results implicate a significant number of unsatisfactory results, especially those referring to microbiological status of food contact surfaces and kitchen utensils. Majority of them were from cafe bars, restaurants and small food premises implicating insufficient hygiene practice in place. Better results were obtained from nurseries and school food premises, but there was still an evident need for better education of the personnel. In both categories the most represented were aerobic mesophilic bacteria, which cause different health problems – from mild to life threatening. Foodborne illnesses is a growing health problem, therefore data related to microbiological status are necessary for food safety risk assessment.

## INTRODUCTION

It is well known that food and water are major sources of bacterial contamination that can cause illnesses. Most of these illnesses can be prevented with careful food handling practices. The major responsibility for ensuring safe food in institutions falls to food service (1). Data from WHO suggest that food-borne disease (together with water) is a significant contributor to mortality from diarrheas disease (2.1 million deaths in 2000). Each year, foodborne disease causes an estimated 76 million illnesses, 325 000 hospitalizations and 5 000 deaths in the United States of America, and 2 366 000 cases, 21 138 hospitalizations and 718 deaths in England and Wales. A recent OECD (Organisation for Economic Co-operation and Development) report assumed that the burden of food-borne disease is probably similar in most OECD countries. Many countries, including developing countries, lack strong surveillance and reporting systems and therefore statistical estimates are not available. Furthermore, food-borne disease often goes unreported, with the result that the economic and health impacts are greater than the figures suggest in many countries. Improvements in the protection of public health rely on improvements in the safety of food. In this regard, governments, the food industry and consumers have a shared responsibility to adopt (2). In order to prevent foodborne disease in Croatia, inspection of food services and premises is mandated by sanitary inspection services which are under the Ministry of health and social welfare. Every year Ministry establishes official sampling plan based on previous inspection results and assessed needs. Despite many advances in food technology, it is still difficult to ensure food safety “from stable to table”, so the most efficient way to produce and serve safe food is to establish programs such as Good Hygienic Practises (GHP) and Good Manufactory Practises (GMP) (3). These measures represent the pre-requisite conditions required in facilities for preparing or manufacturing food. (4) Significant specific hazards are addressed applying the Hazard Analysis and Critical Control Point (HACCP) principles developed more than 40 years ago. HACCP become an increasingly important component of commercial food production practices, which later was found its role in other aspects of food businesses, confirming that can influence on decreasing of food-borne outbreaks. The principles of the Hazard Analysis and Critical Control Point (HACCP) system have been adopted by the Codex Alimentarius Commission and guidelines to its application are provided in an Annex to the General Principles of Food Hygiene (FAO and WHO, 2003) (4). Food Act (OJ 46/07) regulates implementation of HACCP systems in all objects that are dealing with food no later than 1<sup>st</sup> January

2009 (5). The aim of this study was to examine the microbiological contamination of food contact surfaces and kitchen utensils and to determine the acceptability of hygiene levels in small food premises and food services in schools and nurseries in order to assess real situation on the field and be able to influence on its improvement.

## **MATERIALS AND METHODS**

Beside official control, small food premises and food services are controlled according to the contract with county Public Health Institute. Number of testing per year is part of the contract, but mostly it is three or four times over a one year period. During this time, it was examined 3035 samples from 221 food services and premises. For each sampling it has been taken 5 samples (4 swabs of surfaces + 1 swab of hands) according to the swab contact method in order to collect samples from food-contact surfaces or kitchen utensils and hands of the employees. The tip of each sterile cotton swab was moistened with sterile phosphate-buffered saline (pH = 7.0) and then rolled repeatedly over the surface or hands. Finally, all swabs were placed in a labelled test tube containing sterile phosphate-buffered saline using aseptic techniques. After sampling, the handle of the swab was aseptically broken as the swab tip was placed into the test tube and capped. All test tubes were marked with identification mark and sample number. Samples were held in cooler with ice during the transport to Public Health Institute of Osijek – Baranya County. Transportation time to the laboratory was less than 2 hours, and samples were analyzed within the 5 hours of the arrival at the laboratory. All samples were microbiologically analysed in order to determine aerobic mesophilic bacteria according to HRN ISO 4833/2003 method (6), enterobacteriaceae according to HRN ISO 8523/1999 method (7) and Streptococcus D group according to ISO 7899/2001 method (8). The number of colony-forming units in logs ( $\log_{10}$ CFU) were calculated. Results were presented according to the Regulation on normative of microbiological clearness and determining methods (OJ 46/94) (9) in order to assess distribution by comparison with total number of samples, samples origin and frequency regarding the type of bacteria.

## **RESULTS**

Based on obtained results, the total number of positive samples tested on all three microbiological parameters has showed worrying high number (Fig. 1).

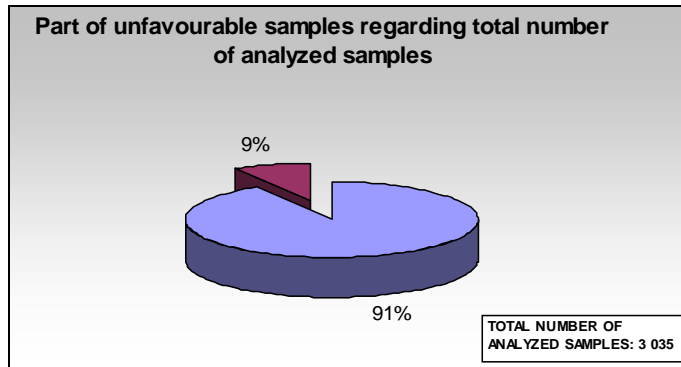


Figure 1. Part of unfavourable samples regarding total number of analyzed samples.

When dividing this number into three groups according to the type of food businesses (schools and nurseries, small food businesses and catering), picture was even more disturbing: more than 40% of positive results were obtained in catering and also significant number in school and nurseries (Fig. 2).

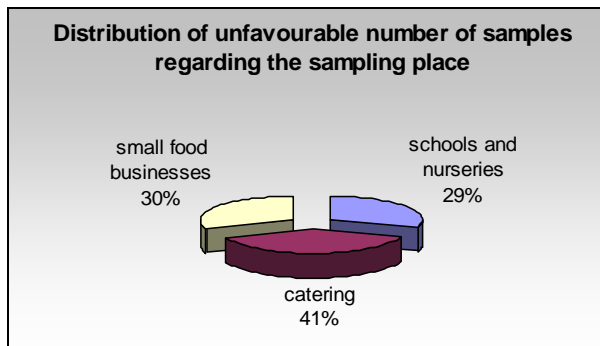


Figure 2. Distribution of unfavourable number of samples regarding the sampling place.

The most common reasons of unfavorable results were almost equally divided between aerobic mesophilic bacteria (52%) and enterobacteriaceae (43%) (Fig. 3).

Unfavourable samples are then divided according to the sampling objects (food-contact surfaces or kitchen utensils and hands of the employees) and chi square a probability was calculated in order to determine significant differences. It can be concluded that food-contact surfaces or kitchen utensils showed significant differences at the level  $p < 0.01$  comparing to admissible microbiological standards. At the same time, results indicate on unsatisfactory hygiene practice in all of three food business facilities.

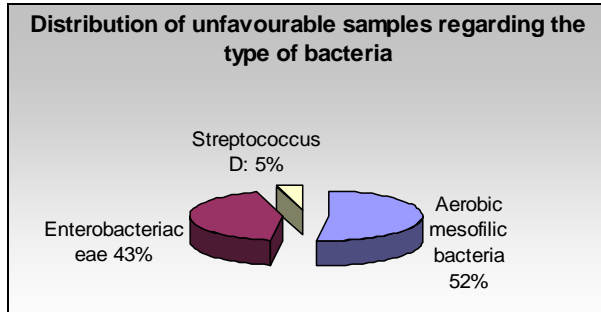


Figure 3. Distribution of unfavourable samples regarding the type of bacteria.

Table 1. Results of unfavourable samples according to the sampling objects.

	Schools and nurseries		Catering		Small food businesses	
	swab of hands (%)	swab of food contact surfaces (%)	swab of hands (%)	swab of food-contact surfaces (%)	swab of hands (%)	swab of food-contact surfaces (%)
<b>Aerobic mesophilic bacteria</b>	9.41	49.41	4.31	52.59	1.14	38.64
<b>Enterobacteriaceae</b>	11.76	27.06	7.76	33.62	9.09	39.77
<b>Streptococcus D</b>	-	2.35	1.72	-	2.27	9.09

## DISCUSSION

There is a growing tendency to eat in places other than home. Because of that fact, the number of restaurants has increased in recent years (10). In the same time, children are spending much more time in school and nursery than at home, so nurseries and schools kitchens became one of the most common places where children eat. In those establishments food is prepared mostly in central kitchen and than distributed to all other establishments. Few American studies that had been conducted over the past two-three years estimated that 55% of food poisoning cases are caused by improper cooking and storage of foods, and 24% because of poor hygiene (e.g. not washing hands before handling food). Only 3% of cases were from an unsafe food source.



This study indicates that public restaurants, as well as school and nursery restaurants, with poor hygiene results may cause an increased risk of foodborne outbreaks. The limited number of researches related to food safety in schools indicates on food-handling problems which should be addressed. The trend of increasing number of school foodborne illness outbreaks demonstrates the need to implement the HACCP in school foodservice. Although the need seems evident, only a small percentage of schools have implemented a HACCP system (11). The other part of the problem is that foodborne illnesses are significantly underreported. Epidemiological surveillance has shown an increase in the prevalence of foodborne illness, therefore the data for United States indicates on approximately 76 million illnesses, 325 000 hospitalizations and 5 000 deaths. For Australia those data differs: 5,4 million illnesses, 17 700 hospitalizations and 125 deaths. In China were estimated 300 million cases of illnesses per year (12). It is obvious that foodborne illnesses presents huge burden to economy in all parts of the world.

To provide safe food and prevent foodborne illness outbreaks, hazard analysis critical control point (HACCP) programs are recommended. The National Advisory Committee on Microbiological Criteria for Foods defined HACCP as 'a management system in which food safety is addressed through the analysis and control of biological, chemical and physical hazards from raw material production, procurement and handling, to manufacturing, distribution and consumption of the finished product' (13), (14). So it is clear that HACCP is a hazard management tool. The "handling procedures description" and "other prerequisites" from the infrastructure within the HACCP procedure can be developed and implemented. Prerequisites can both, simplify HACCP procedure development and facilitate procedural effectiveness. One of the prerequisite of HACCP is development of SOPs to address non-specific food safety issues. However, specific recommendation may be made with regards to the safe handling or processing of certain foodstuffs (15).

The other part of this paper results indicates that hygiene of kitchen utensils and food contact surfaces shown higher number of unsatisfactory results than personal hygiene of the staff. The reasons might be either because of insufficient or improper sanitation, or insufficient knowledge of food safety practices. Nevertheless, the retention of bacteria on food contact surfaces increase the risk of the cross-contamination of these microorganisms to food. The risk may be lowered when the surfaces are dry, partly because bacterial growth and survival would be reduced. Some study highlights the facts that pathogens remain viable on dry stainless steel

surfaces and present a contamination hazard for considerable period of time, dependent on the contamination level and type of the pathogen. Systematic studies on the risk of pathogen transfer associated with surface cleaning when using contaminated dishes provide quantitative data from which a model of risk assessment could lead (16).

In order to help implementing HACCP in food business facilities, government agencies role are both, a strategic one – in the implementation of HACCP, as well as an operative one – in organizing the effective and ongoing assessment of HACCP systems. A key role of government agencies will be to show leadership by promoting and facilitating the implementation of HACCP (17). The types of activities that government agencies need to consider have been described in different FAO and WHO documents. In summary, these roles could include:

- facilitating training programmes for personnel;
- providing necessary infrastructure in terms of guidance, expertise & where appropriate, legislation;
- encouraging the necessary support and development of training material;
- formulating an overall programme to assess HACCP systems.

In addition, government agencies have a duty to clearly communicate all health and safety standards, regulations, guidelines, and other requirements. However, the governments should always remain accountable for food safety (18).

## **CONCLUSION**

- High number of unsatisfactory results is implicating on urging need of implementation of HACCP systems in all food business facilities.
- Sanitation standard operating practises need to be developed and well maintained. This also includes proper hygiene standards.
- Training programs for personnel should be provided in order to increase awareness of hygienic importance regarding food handling and food operating.
- Enforcement of monitoring regarding different types of food services will be advisable in order to get broader picture of this problem and for the purposes of better official control programs. Based on larger number of data it would be also possible to make risk assessment.

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# EVALUATION OF CLEANLINESS IN DAIRY PLANTS AND INNOVATIONS FOR IMPROVING HYGIENE

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**Introduction:** Hygiene has an important role in food industry to produce healthy and high quality products. Therefore, effective cleaning systems, hygienic design and good manufacturing practices are irreplaceable to keep high hygiene level in dairies. The literature part of the study gives overview about microbial ecology in dairies. Also sampling and detection methods are described. In addition basic cleaning methods and also innovative cleaning techniques like ultrasound, ozone and UV cleaning methods used in dairies are introduced. This study also presents information about common hygiene problems and cleaning procedures in dairies. The aim of the experimental part of this study was to evaluate cleanliness of dairy plants and innovations for improving hygiene. Therefore, hygiene level was investigated before and after improvement in the hygiene practices in four Estonian dairies. Methods: To make improvement dairies had used ultrasound equipment for cleaning of small utensils and personnel had practical hand hygiene course.

**Methods:** Microbiological samples were taken from surfaces with self-made contact plates, commercial contact agars, non-woven cloths and *Listeria* Isolation Transport swabs. Also air samples, water samples, raw material and product samples were taken. Temperature, humidity and pH were measured and pictures were taken at each sampling point. In this study aerobic count bacteria, yeasts and moulds, *Enterobacteriaceae*, Coliform, *Escherichia coli*, *Listeria* spp., *Listeria monocytogenes* and *Bacillus cereus* were detected using culturing methods. Besides, of traditional cultivation *L. monocytogenes* was also detected using commercial real time PCR kit for *L. monocytogenes*. In addition, disinfection efficiency of ozone technique was investigated in laboratory study.

**Results:** The results of these surveys indicated that hygiene level and cleaning results did not improve between two samplings. Cleaning methods used in dairies were effective to provide adequate cleanliness of direct product contact surfaces. Cleanliness of indirect product contact surfaces and environmental surfaces could be improved. Comparison of water samples with surfaces showed that water quality affected cleanliness of surfaces. Results of detecting pathogens from dairies demonstrated that cleaning method used in dairies should be more effective to minimize the potential risk of pathogen contamination. Air samples demonstrated quite high aerobic bacteria and yeasts and mould counts. Results of ozonation test indicated that ozonation is effective method to destroy *L. monocytogenes* from cloths. Conclusion: Cleaning systems used in dairies need improvements to increase the hygiene level in dairy plant and to be able to product safe and high quality products.

# ASSESSMENT OF THE SANITATION EFFICACY IN THE ESTONIAN MEAT INDUSTRY

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Cleanliness is clearly essential in preventing product contamination, especially because many pathogens that survive the cleaning/sanitizing process may be attached to or growing on food contact surfaces. The main **aim** of the current study was to analyze the sanitation efficacy in Estonian large-scale meat industry using three different surface hygiene assessment methods. The second aim was to identify the possible control points for the control of sanitation efficacy, and practicality of the hygiene measurement methods used within the self-control programs of the meat industry.

**Methods** used were aerobic plate count (APC), adenosine triphosphate bioluminescence (ATP, Clean-Trace®) and traditional microbiological swabbing method. It can be stated that the APC do not measure the entire bacterial population, but rather the number of bacteria that grow in the presence of oxygen (aerobically) at medium range (mesophilic) temperatures. The APC can be used to evaluate the sanitary condition of a food product or equipment, if performed after sanitation, it can be used to measure the effectiveness of the cleanup process. The ATP method is rapid hygiene monitoring technique which is based on the detection of adenosine triphosphate by bioluminescence. ATP measurement was performed in accordance with manufacturer instructions. The result, the amount of light produced, is directly related to the level of microbial and non-microbial ATP present in the sample and is often referred to as the hygienic status of the sample. ATP hygiene testing is allowing corrective action to be taken prior to the processing of product and identifies the presence of product and microbial residues that are not visible on plant surfaces. ATP bioluminescence indicates direct and indirect risks. In the swabbing method the surface area of interest is swabbed with a cotton-tipped stick to collect possible microorganisms. The collected microorganisms are then released

into an appropriate solution for subsequent cultivation. The advantage of swabbing method was that it provides good access in confined process areas. A total of 392 samples were taken and analyzed during November 2006 – February 2007. All samples were taken in high-risk processing areas from where the risk of spreading contamination to food products from surfaces next to surfaces with food contact was existing, mainly from areas of chopped and ground meat packaging, ready-to-eat products slicing and packaging. The industry inner standard for aerobic plate count for clean surfaces in high hygiene level areas was  $< 50 \text{ CFU}/24 \text{ cm}^2$ . For conventional swabbing method it was  $\leq 50 \text{ CFU}/25 \text{ cm}^2$ , and for ATP swab test it was  $< 500 \text{ RLU}/10 \text{ cm}^2$ . The APC and swabbing methods are the tools for measuring the aerobic bacterial counts from production surfaces. The ATP method is used for hygiene status measurement on surfaces where no organic matter after sanitation should be found and it can be especially useful for the measurement of 'total hygiene' in specified control points of present large-scale meat industry.

All **results** were divided to have acceptable i.e. 'clean' or unacceptable i.e. 'dirty' levels in accordance with industry inner standards. The not acceptable surface hygiene results with APC, ATP and traditional swabbing were registered in 10%, 17% and 5% of samples, respectively. When using three different methods in the measurements 76% of the results were similar (all gave acceptable or not acceptable results) and 24% were non-comparable. Main differences were observed with samples giving unacceptable results i.e. with the classification of 'dirty' surfaces. Most similar results (91.4%) were registered within APC and traditional swabbing methods. There was more non-similarities when the results of ATP measurement were compared with the results of APC and conventional swabbing method. It can be explained with the differences in methods used.

As a **conclusion**, it was found that all three hygiene assessment methods were useful tools within self-control program of the large-scale meat industry but there is need for combination of all methods for proper surface hygiene monitoring. There is need to look how the sanitation programme is performing over a longer defined time period (quarterly, yearly) in present meat industry to ensure that the programme remains within control and to try to improve the programme performance. In present large-scale meat industry the control points were appointed and changes in sanitation procedures were performed.

# IMPACT OF FOOD PROCESSING AND STORAGE CONDITIONS ON THE NITRATE CONTENT IN CANNED VEGETABLE-BASED INFANT FOODS

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**Background:** The nitrate and nitrite contents were determined in canned vegetable-based infant foods of five varieties. Vegetable based infant foods investigated in this study form an essential part of infant foods consumed in Estonia. Furthermore, changes in nitrate content during industrial processing were studied. Samples were taken from raw material, homogenized mixture and final product after sterilization. Furthermore, the effect of storage time (24 and 48 h) and temperature (4–6°C and 20–22°C) on nitrate and nitrite content was studied in 6 different products. **Results:** During processing the nitrate content in canned infant foods decreased 39–50% (average 45%). After 24 h storage at refrigerated and room temperatures the mean nitrate content increased in average 7% and 13% and after 48 h storage 15% and 29%, respectively. **Conclusion:** Nitrate content in final products was related to the nitrate contents in raw material. Significant decrease of nitrate during infant food processing was registered. The opened infant food cans should only be stored at refrigerated temperatures and consumed during a short period.



# MODELLING OF *STAPHYLOCOCCUS EPIDERMIDIS* ADHERENCE TO STAINLESS STEEL IN A MILK FLOW SYSTEM

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Secondary contamination of both raw milk and milk products could be due to biofilm-forming microorganisms occurring on the contact surfaces of the processing plant equipment. These microorganisms can belong to a pathogenic species such as the clinically significant species *Staphylococcus epidermidis*, which pathogenicity primarily consists in its ability to colonize indwelling polymeric devices and to form a thick, multilayered biofilm. Initial adherence of microorganisms to contact surfaces is a precondition for bacterial biofilm formation and the present study investigated the adherence of *S. epidermidis* to stainless steel under experimental conditions. We set up a device in which cow milk, contaminated with a milk-related biofilm-positive *S. epidermidis* isolate, flowed through special chambers containing stainless coupons over the course of 6 h. The experiment was performed under two flow rates corresponding to two levels of laminar flow (Reynolds numbers  $Re = 9.38$  and  $Re = 31.33$ ) and under static conditions. Three different temperatures (22, 25 and 28°C) of milk were tested during the experiment. The effects of flow rate and temperature of milk on *S. epidermidis* adherence were analysed. Under all conditions tested, the highest adherence occurred during the first 2 h and it was observed only on the upper side of the stainless steel coupons. A higher and persisting adherence of *S. epidermidis* was observed if a temperature was just below the growth range (22°C). Under lower temperatures (22 and 25°C) no significant influence of milk flow on the adherence was observed when compared with static conditions. However, at the milk temperature of 28°C, when the culture switched from the lag phase to the early exponential growth phase (at about the time point of 2 h), the cells almost completely detached from the coupons' surfaces but only under dynamic conditions

whereas under static conditions the cells remained attached. We assume that the transition from one growth phase to another was accompanied by changes in the cell surface qualities and due to the fact that milk was flowing such the cells could easily be washed away. The results of the present study showed that the adherence of *S. epidermidis* occurred within first two hours and was highly influenced by temperature and gravitation. It follows from the results that suboptimal growth temperatures and long horizontal transport systems are heavily involved in the initial adherence, whereas higher temperatures enabling growth may lead to the cell detachment. The rate of the milk flow did not seem to be a factor significantly affecting the adherence of the cells. Due to the fact that biofilms can be formed on contact surfaces in food processing plants, it is very important to recognize the conditions which may influence their formation and persistence. This study was supported by the projects of Ministry of Agriculture of the Czech Republic (QF4048 and MZe00027162).

## APPENDEIX 1: PARTICIPANT LIST

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45. Gun Wirtanen, VTT, Espoo, Finland



## APPENDIX 2: PROGRAMME

Arrival on Sunday evening – information event at the Hotel Ansgar

### Monday, October 13, 2008 – Theory

7.30	The bus leaves Hotel Ansgar and drives to DTU
8.15 – 8.30	Registration at the DTU
8.30 – 8.40	Welcome by SAFOODNET project coordinator, Gun Wirtanen, VTT, Finland and Alan Friis, DTU National Food Institute, Denmark
8.40 – 9.20	Practical aspects of plant design, Tony Hasting, Tony Hasting Consulting, UK
9.20 – 9.45	Influence of fluid flow and CFD in closed processes, Bo Boye Busk Jensen, Alan Friis, DTU National Food Institute, Denmark
9.45 – 10.10	Industrial and analytical methods for the detection of industrial food fouling, Kathryn Whitehead, Manchester Metropolitan University, UK
10.10 – 10.50	Coffee/tea break and introduction to task 1
10.50 – 11.20	Microbial issues in fish factories – how to use disinfection ? Birthe Fonnesbech Vogel, DTU Aqua, Denmark
11.20 – 11.40	Testing of disinfection efficacy, Gun Wirtanen, VTT, Finland
11.40 – 11.50	<i>Break</i>
11.50 – 12.10	Commercial disinfectants and their applications, Lars Mikkelsen, Ecolab, DK
12.10 – 12.30	Food Safety in practice, Eigil Pedersen, Bactoforce, Denmark
12.30 – 13.10	<i>Sandwich lunch at DTU</i>
13.10 – 13.30	Introduction to task 2

13.30 – 14.00	Predictive microbiology and application of mathematical models, Paw Dalgaard, DTU Aqua, Denmark
14.00 – 14.10	<i>Break</i>
14.10 – 14.30	HACCP systems, Tina Beck Hansen, DTU National Food Institute, Denmark
14.30 – 15.00	HYGRAM® as a decision support tool, Savvas Gennaris, visiting scientist at VTT, Finland/Cyprus.
15.00 – 15.40	<i>Coffee/tea break</i> and introduction to task 3
15.40 – 16.45	Preparation of presentation
16.45 – 17.00	Detection of <i>Salmonella</i> in Chicken Meat by LightCycler PCR, Aysegül Eyigör & Seran Temelli, Uludag University, Turkey
17.00 – 18.30	<i>Dinner at DTU</i>
18.30 – 18.50	Presentation of task 1
18.50 – 19.10	Presentation of task 2
19.10 – 19.30	Presentation of task 3
19.30 – 21.30	Get together and Bus to hotel Ansgar in Copenhagen starting at 21.30

## **Tuesday, October 14, 2008 – Practical work**

7.50	Bus from Hotel Ansgar to DTU
8.45 – 12.30	<u>Practical sessions in building 227 room 150:</u> Each task starts with 15 minutes of presentation Team 1 does exercise 1 Team 2 does exercise 2 Team 3 does exercise 3
12.30 – 13.15	<i>Sandwich lunch</i>
13.15 – 17.00	<u>Practical sessions:</u> Each task starts with 15 minutes of presentation Team 1 does exercise 2



Team 2 does exercise 3  
Team 3 does exercise 1  
17.15 Bus to Tivoli in Copenhagen  
18.00 *Dinner at Restaurant HerceGovina* in Tivoli and free time

## **Wednesday, October 15, 2008 – Continuation of the practical session**

7.50 Bus from Hotel Ansgar to DTU  
8.45 – 12.30 Continuation of practical session in building 227 room 150  
Each task starts with 15 minutes of presentation  
Team 1 does exercise 3  
Team 2 does exercise 1  
Team 3 does exercise 2  
12.30 – 13.15 *Sandwich lunch*  
13.15 – 14.15 Wrap up lectures:  
- Practical example of process hygiene in Estonian dairys,  
Raivo Vokk, Technical University of Tallinn, Estonia  
- State of art in hygienic design in food industry,  
Alan Friis, DTU National Food Institute, Denmark  
14.15 – 14.25 Summary of the workshop, Hanne Løje, DTU National  
Food Institute, Denmark  
14.25 – 14.40 *Coffee/tea break*  
14.40 – 16.10 Exam based on multiple choice questions and questions  
with answers to be listed  
16.15 -> Departure with taxi to the airport and bus to the hotel





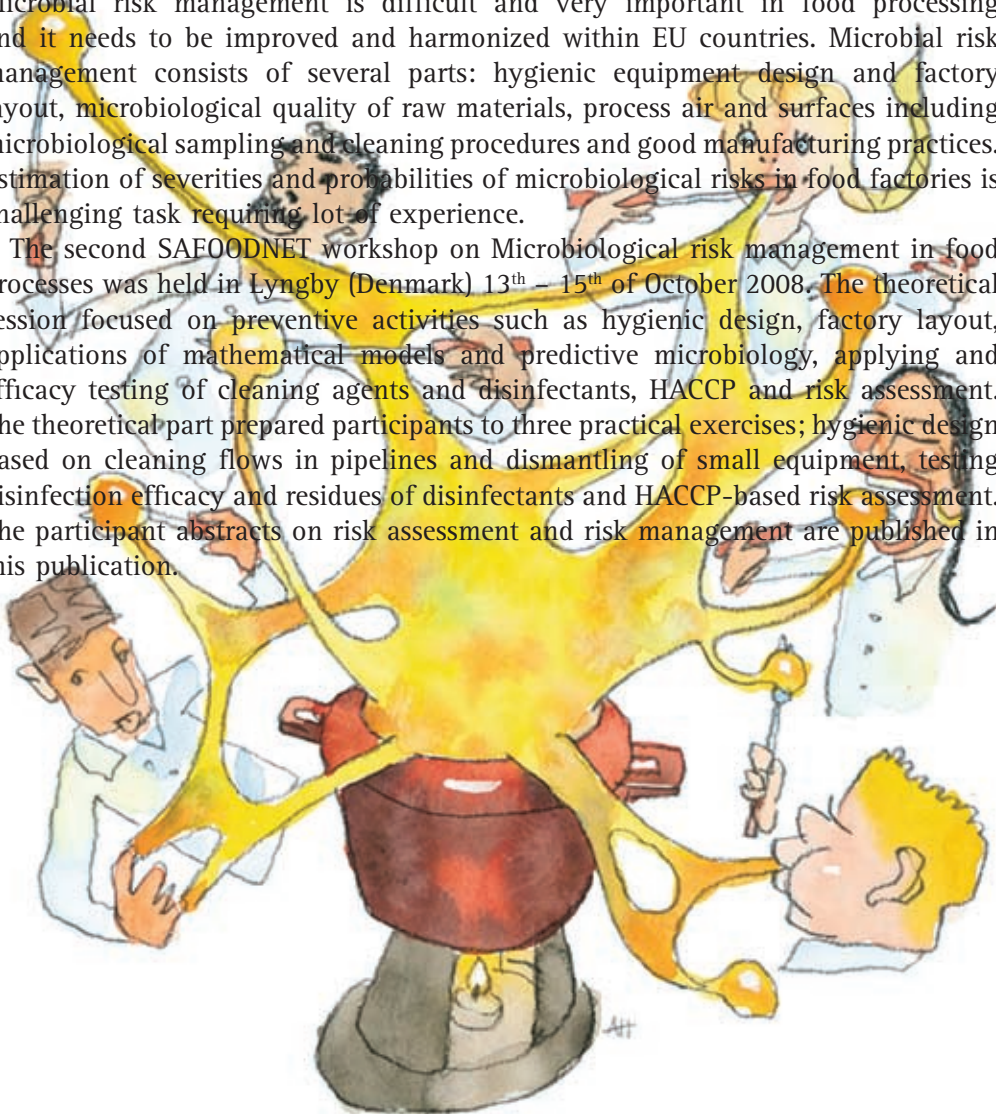
Series title, number and report  
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VTT Symposium 260  
VTT-SYMP-260

Author(s) Gun Wirtanen & Satu Salo		
Title <b>MICROBIAL RISK MANAGEMENT IN FOOD PROCESSES</b>		
Abstract Microbial risk management is difficult and very important in food processing and although we have common EU legislation for food producers microbial risk management needs to be improved and harmonized within EU countries. Microbial risk management consists of several parts. Knowledge of hygienic design and factory layout can prevent severe microbial hazards. Virtual simulations and modelling can help in testing and improving the hygienic design of complex food processing lines. Microbiological sampling from large and closed process equipment is challenging. Choosing suitable cleaning agents and disinfectants for various food processing equipment and process environment requires knowledge about efficacy of chemicals and properties of surface materials. Estimation of severities and probabilities of microbiological risks in food factories is dependent on the experience of the person doing the estimation. Decision support tools such as HYGRAM <sup>®</sup> can be used for making risk assessment in food factories. The workshop on <i>Microbiological risk management in food processes</i> was held in Lyngby (Denmark) 13 <sup>th</sup> –15 <sup>th</sup> of October 2008. The theoretical session focused on preventive activities such as hygienic design, factory layout, applications of mathematical models and predictive microbiology, applying and efficacy testing of cleaning agents and disinfectants, HACCP and risk assessment. The theoretical part prepared participants to three practical exercises; hygienic design based on cleaning flows in pipelines and dismantling of small equipment, testing disinfection efficacy and residues of disinfectants and HACCP-based risk assessment. The participant abstracts are also published in this publication.		
ISBN 978-951-38-6343-2 (soft back ed.) 978-951-38-7584-8 (URL: <a href="http://www.vtt.fi/publications/index.jsp">http://www.vtt.fi/publications/index.jsp</a> )		
Series title and ISSN VTT Symposium 0357-9387 (soft back ed.) 1455-0873 (URL: <a href="http://www.vtt.fi/publications/index.jsp">http://www.vtt.fi/publications/index.jsp</a> )		Project number
Date October 2009	Language English	Pages 88 p. + app. 6 p.
Name of project SAFOODNET		Commissioned by SAFOODNET; FP6-022808-2006
Keywords microbial risk assessment, food processing, preventive activities, corrective actions, critical control points, HACCP, Good Management Practice, GMP, harmful microbes, pathogens, microbiological risk management, equipment design, surface materials, layout, cleaning, disinfection, contamination routes, monitoring, product quality, sampling, training, documentation		Publisher VTT Technical Research Centre of Finland P.O. Box 1000, FI-02044 VTT, Finland Phone internat. +358 20 722 4520 Fax +358 20 722 4374

Microbial risk management is difficult and very important in food processing and it needs to be improved and harmonized within EU countries. Microbial risk management consists of several parts: hygienic equipment design and factory layout, microbiological quality of raw materials, process air and surfaces including microbiological sampling and cleaning procedures and good manufacturing practices. Estimation of severities and probabilities of microbiological risks in food factories is challenging task requiring lot of experience.

The second SAFOODNET workshop on Microbiological risk management in food processes was held in Lyngby (Denmark) 13<sup>th</sup> – 15<sup>th</sup> of October 2008. The theoretical session focused on preventive activities such as hygienic design, factory layout, applications of mathematical models and predictive microbiology, applying and efficacy testing of cleaning agents and disinfectants, HACCP and risk assessment. The theoretical part prepared participants to three practical exercises; hygienic design based on cleaning flows in pipelines and dismantling of small equipment, testing disinfection efficacy and residues of disinfectants and HACCP-based risk assessment. The participant abstracts on risk assessment and risk management are published in this publication.



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