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PAPER

Microbial hazards in food

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Next to bacteria microbial hazards in food comprise mycotoxin-producing moulds, protozoae, viruses and prions. Epidemiological aspects are mentioned which are essential for the pathogenesis of foodborne diseases caused by these agents. Compared with chemical and physical hazards the occurrence or emergence of microbiological hazards depends on a variety of factors and influences during food production and processing that well structured hygiene and food safety management concepts respectively are essential.

In particular this paper refers to the minimal infective dose for man, the factors influencing growth of bacteria in food and to predictive microbiology. The application of the hazard analysis critical control point (HACCP) concept for microbial hazards is mentioned with particular emphasis on the importance of monitoring.

Due to the particular significance of the faeco-oral transmission route for many bacterial foodborne diseases basic hygiene measures assume a decisive importance in food safety management. The HACCP system is based upon such measures and should only be viewed as part of an efficient total hygiene concept in food companies. The example of drinking milk pasteurization illustrates that the introduction of the HACCP system does not constitute a fundamentally new development and that it should rather be viewed as a renaissance of old principles of natural science and hygiene.

Keywords: HACCP; microbial hazards; minimal infective dose; predictive microbiology; basic hygiene measures

INTRODUCTION

Within the hazard analysis critical control point (HACCP) system there is a distinction between biological, chemical and physical hazards. Biological hazards (*Figure 1*) can either be macroparasites or microorganisms. Next to bacteria microbial hazards in food there are various eucaryotic microorganisms like fungi or protozoae (e.g. *Toxoplasma*, *Sarcocystis* species, *Cyclospora*, *Giardia* and *Cryptosporidium*). Furthermore, they comprise viruses and prions. Protozoae, viruses and prions cannot multiply in food. They are either present in raw food of animal origin like meat or brought into the food by way of contamination. In contrast, with the exception of individual species fungi and bacteria can multiply in food, if certain conditions which are necessary for their growth are met.

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VIRUSES

Among virus species for whom foods can serve as vectors (Adams and Moss, 1995) there are basically the following: poliovirus; hepatovirus (hepatitis A) and various gastroenteritis viruses like rotavirus; astrovirus as well as caliciviruses which include Norwalk and Norwalk-like viruses (Cliver, 1990). Man is the reservoir for these agents. Transmission is essentially via the faeco-oral route. Here, foods can play a role as vectors. The foods can either be contaminated directly by man or indirectly by way of contaminated water. The consumption of raw shellfish or oysters led to various outbreaks of hepatitis A

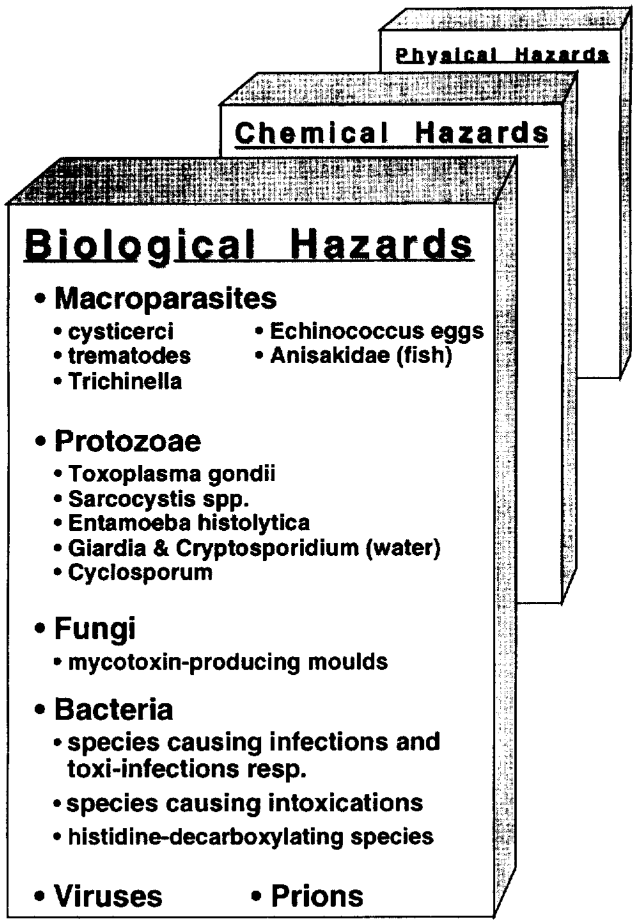

Figure 1 Hazards in food

and gastroenteritis. The tenacity of the mentioned virus species towards heating procedures varies. The hepatitis A virus is the most resistant of those viruses. Raising the internal temperature of shellfish to 85–90°C for 1 min reduces the level of infectious hepatitis A virus > 10 000-fold (ICMSF, 1996).

An effective prevention is initially provided by strict compliance with basic hygiene measures. The hygiene behaviour of man plays an important part in this context. It must be ensured that pathogens cannot be passed directly onto foods from infected persons or indirectly via water.

It also includes the requirement to grow and harvest oysters and shellfish only in regions that are free of human sewage. Moreover, further cross-contaminations between contaminated and uncontaminated foods must be safely excluded.

PRIONS

Prions are considered to be the cause of bovine spongiform encephalitis (BSE). The transmission of this infectious agent onto man through the consumption of beef is assumed but not proven as yet. The risk of transmission via muscle tissue is considered to be low. In contrast, brain, spinal chord, thyroid gland, spleen and the large lymph nodes are provisionally not released for human consumption if the slaughtered animals come from regions where BSE can be found. The tenacity of the infective agent is extremely high. Even heating procedures that are used for a botulinum cook in cans do not inactivate the agent. This epidemiological situation leaves no possibility for establishing suitable control measures in the context of the HACCP system, for example, in a meat processing company.

PROTOZOA

The infection of humans with *Sarcocystis* spp. and *Toxoplasma gondii* occurs mainly via ingestion of raw or insufficiently heated meat which contains mature cysts of these protozoa. *Sarcocystis hominis* is transmitted by beef and *Sarcocystis suihominis* by pork (Fayer, 1994). In contrast, mature *Toxoplasma* cysts can be ingested with the infected meat of various domestic and wild animal species. In addition, with this agent the direct faeco–oral route from cat to man is of significance (Jacquier *et al.*, 1995). Mature cysts of both protozoa in meat do not survive temperatures > 70°C. They are also killed within 3 days at −20°C. In meat which is stored at +4°C they can survive up to 3 weeks.

The infection of humans with *Entamoeba histolytica*, *Giardia lamblia* and *Cryptosporidium parvum* occurs via the faeco–oral route. In the case of *Giardia* and *Cryptosporidium* drinking water is the most important epidemiological vector. Apart from man, animals can also excrete cysts or oocysts from these agents. In the case of *E. histolytica* man is the most important source of infection. In contrast, with *Cryptosporidium* various animal species, in particular calves, are important pathogen reservoirs. *G. lamblia* is also widespread with animals. However, it remains to be found out whether cysts from animals are of significance for infections in humans (Eckert, 1993).

Cyclospora infections are usually seen in connection with cases of travel diarrhoea. Here the faeco–oral route of transmission from man to man should equally be the most significant one (Gascon *et al.*, 1995). To begin with, immunocompromised persons were reported to have fallen ill. More recently, also immunocompetent persons were affected.

It goes without saying that with faeco–oral transmission basic hygiene measures, which have already been referred to in the context of viruses, have to be considered as essential preventive measures. It has to be emphasized that the chlorination of drinking water, which is commonly used, does not inactivate cysts of *E. histolytica* and *G. lamblia* nor oocysts of *Cryptosporidia*.

FUNGI

Fungi which have to be mentioned in connection with foodborne diseases are mycotoxin-producing moulds.

Not the organisms themselves but their mycotoxins are toxigenic for humans and have to be considered as hazards. Mycotoxins are produced by moulds in raw materials or in foods. However, they can also be ingested by animals via feed and are then excreted, for example, into milk. Aflatoxin B_1, for example, is ingested by cattle and is passed into milk as aflatoxin M_1. Mycotoxins are low molecular substances which are very resistant against external influences. Thus, they cannot be destroyed reliably by currently used heating procedures.

BACTERIA

Bacteria include species which cause illness by their toxins. These toxins can be heat-labile (e.g. *Botulinus* toxin) or heat-stable (e.g. *Staphylococcus* enterotoxins). Infectious pathogens, however, play a more important epidemiological role. The special significance of bacteria as microbial hazards in food is due to their complex kinetics of inactivation, survival and growth. Hence, the elaboration of HACCP concepts for bacterial hazards requires particularly differentiated deliberations. An essential precondition is to know whether the growth of an organism in food is significant for the emergence of a disease. This point is closely linked to the question of the minimal infective dose (MID) which shall be dealt with. With a low MID and with foods acting merely as vectors, preventive hygiene measures are firstly concerned with preventing contamination of raw materials and of food products during processing. In case it is not possible to ensure this prevention with enough certainty an additional processing step has to be planned in order to inactivate the agent. With bacteria such steps usually consist of a heating process.

Figure 2 contains an overview on bacteria which are found in connection with foodborne diseases (Untermann, 1993). Pathogens which have other routes of infection besides the oral one are figured separately on the left. For the transmission of, for example, *Mycobacterium bovis* and *Brucella melitensis* to humans milk plays an important role while the risk of infection with *Coxiella burnetii* in milk is low and thus other infection routes are considerably more important.

Salmonella typhi, *S. paratyphi* and *Vibrio cholerae* for whom man is the only reservoir cannot multiply in food. Therefore, food figures only as a vector. For *Shigella* spp. the reservoir is also confined to humans. In the case of *Campylobacter jejuni/coli* the decisive pathogen reservoir is poultry. Besides the transmission from man to man contaminated raw chicken carcasses should play a special part during infection because pathogens can be spread onto other foods in kitchens via cross-contamination, for example, by thawing water.

In the USA beef is considered to be the natural reservoir for serotype O157 H7 of verotoxinogenic *Escherichia coli* (EHEC). Hence, the consumption of raw or insufficiently heated meat products is seen as an important source of infection (Matthews *et al.*, 1997). In Europe, the significance of cattle as an important reservoir for EHEC strains has so far not been confirmed (Burnens *et al.*, 1995; Bülte *et al.*, 1996). As with all pathogens with a low MID the faeco–oral route of transmission of verotoxinogenic *E. coli* from man to man plays an important part in the spreading of outbreaks, that is, food products are mainly contaminated by human excretors. Correspondingly, the compliance with basic hygiene measures is a prerequisite for effective prevention.

In contrast, with the remaining infective agents their growth in food is of major epidemiological significance. The same applies to pathogens which strictly cause intoxications because the formation of toxinogenic metabolites in food requires previous microbial growth of such pathogens. Under such conditions, all measures which contribute towards a safe prevention of microbial growth in food constitute an additional

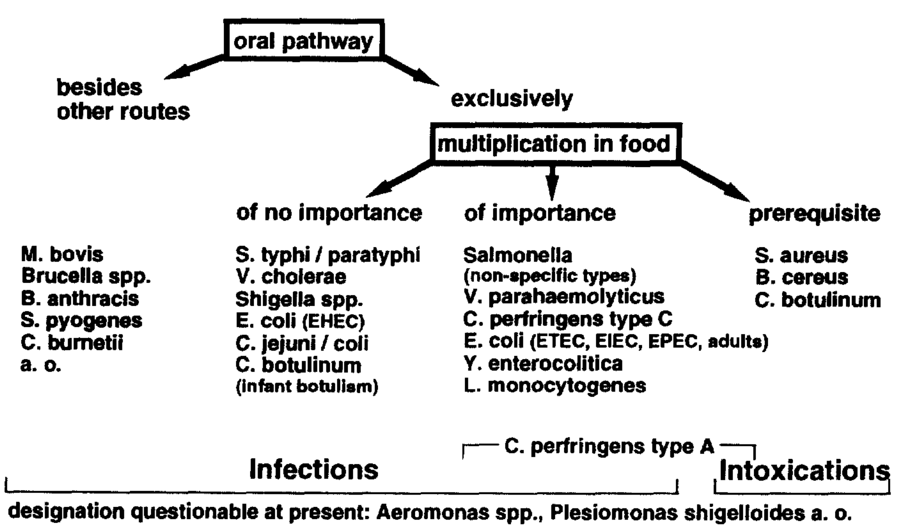

Figure 2 Foodborne bacterial infections and intoxications

safety barrier. *Clostridium perfringens* is situated between infectious and toxi-infectious agents on the one hand and strictly intoxicating agents on the other hand, respectively. The symptoms are caused by an enterotoxin which is produced during sporulation of *C. perfringens* in the small intestine of humans. As a precondition the pathogens must have been ingested together with the food in large numbers and must also have been subject to microbial growth in the small intestine.

HAZARD ANALYSIS

In order to conduct a hazard analysis for microbial hazards as well as for any other hazards it is fundamental to ask the following question: can pathogens be present in raw materials or additives and are contaminations possible during the production process? Then it has to be examined whether during the production process, during storage or during the intended use of the product growth of pathogenic microorganisms or the formation of toxigenic substances, for example, nitrosamines, can occur.

The risk assessment of microorganisms is fraught with particular difficulties. Quantitative scientific risk assessments of microorganisms in food on the basis of the dose–response relationship and exposure assessment as they are customarily carried out for chemical contaminants have so far only been available for *G. lamblia* and for viruses in drinking water (Gerba and Haas, 1988; Haas, 1993; Haas *et al.*, 1993; Macler and Regli, 1993; Notermans *et al.*, 1995; Regli *et al.*, 1991; Rose and Gerba, 1991, Rose *et al.*, 1991). Furthermore, a corresponding study on virus contaminations of mussels and oysters in connection with coastal waters has been published (Rose and Sobsey, 1993).

Two particular difficulties have to be mentioned for the quantification of microbiological hazards caused by the consumption of foods: the determination of the MID; and the complex kinetics of bacterial survival, growth and death in foods.

MINIMAL INFECTIVE DOSE

For most bacterial species the question of the MID cannot be answered satisfactorily. Firstly, it must be borne in mind that among consumers there are special risk groups, like small children, senior citizens, pregnant women and immunocompromised persons. Furthermore, there are various physiological factors that influence the MID, for example: the degree of stomach fluid acidity; the quantity of stomach contents; the intestinal flora; and last but not least, the immunological status of the person. This status is again influenced by immunity due to previous infections, by the nutritional state and by stress, etc. (Herbert and Cohen, 1993).

An additional factor is that, as opposed to chemical residues, the quantity of microorganisms in foods is subject to permanent changes. The complex kinetics of bacterial survival, growth and death in foods are determined by manifold factors which can be differentiated into intrinsic, extrinsic and process factors. They include the pH value, the a_w value, the redox potential, the temperature and the competitive microbial flora. The most important factors which can influence microbial growth in foods are shown in *Figure 3*. Hence, the risks resulting from microorganisms concerning food hygiene and safety and especially from bacteria vary depending on the composition of food, on production, processing and preparation procedures as well as on packaging and storage conditions.

PREDICTIVE MICROBIOLOGY

The synergy of these different factors is very complex. Computer-based mathematical models have been created that take into account the factors previously

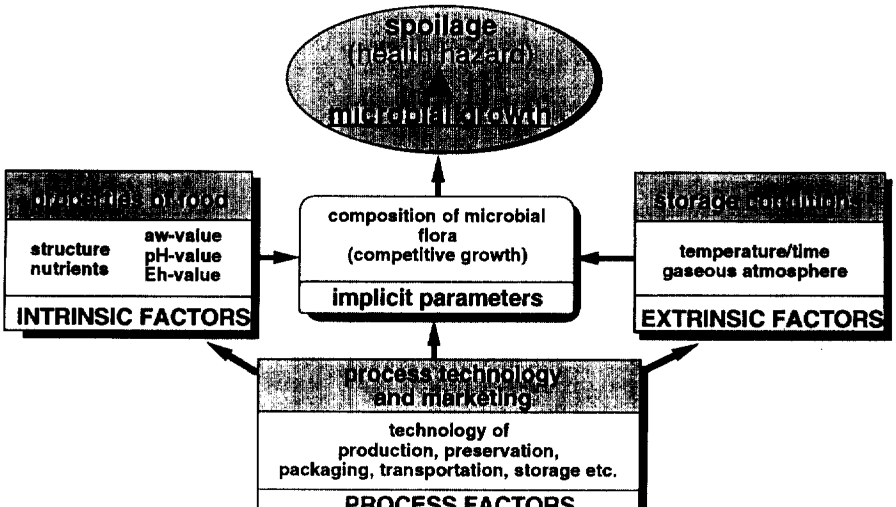

Figure 3 Influences on microbial growth in foods

mentioned. They allow for predictions being made concerning microbial kinetics in foods (Baranyi and Roberts, 1994; Ross and McMeekin, 1994). This *predictive microbiology* is based on data obtained from broth cultures under standardized conditions. If such a programme is fed with data on a food's intrinsic factors, on the atmospheric conditions of packaging and on the projected storage temperature and storage time, the prospective behaviour of a microbial species can be computed and depicted in graphs.

However, due to the complex composition of foods results from predictive microbiology can only provide a framework for the understanding of the ecology and kinetics of microorganisms in foods. In order to obtain exact values and figures for a particular food further testing is necessary. *Storage trials* are a suitable means to test the behaviour of certain microorganisms as long as the species in question can be found regularly in the respective food product. If this is not the case *challenge tests* can be performed where foods or raw materials are spiked with the pathogens (Notermans *et al*., 1993).

PREVENTIVE MANAGEMENT

The basic principles of the HACCP system are summarized in *Figure 4*. The particular objective of this figure is to stress that effective controlling and reliable monitoring are preconditions for a critical control point (CCP). It is of little use to introduce further subdivisions into various CCP categories, as has been suggested, for example, by Bryan (1996). It is essential that the safety objective which has been determined according to the hazard analysis is complied with effectively and reliably. The effectiveness is determined by control measures while the reliability is ensured by monitoring.

Apart from very few exceptions rapid microbiological methods cannot be used for monitoring in the

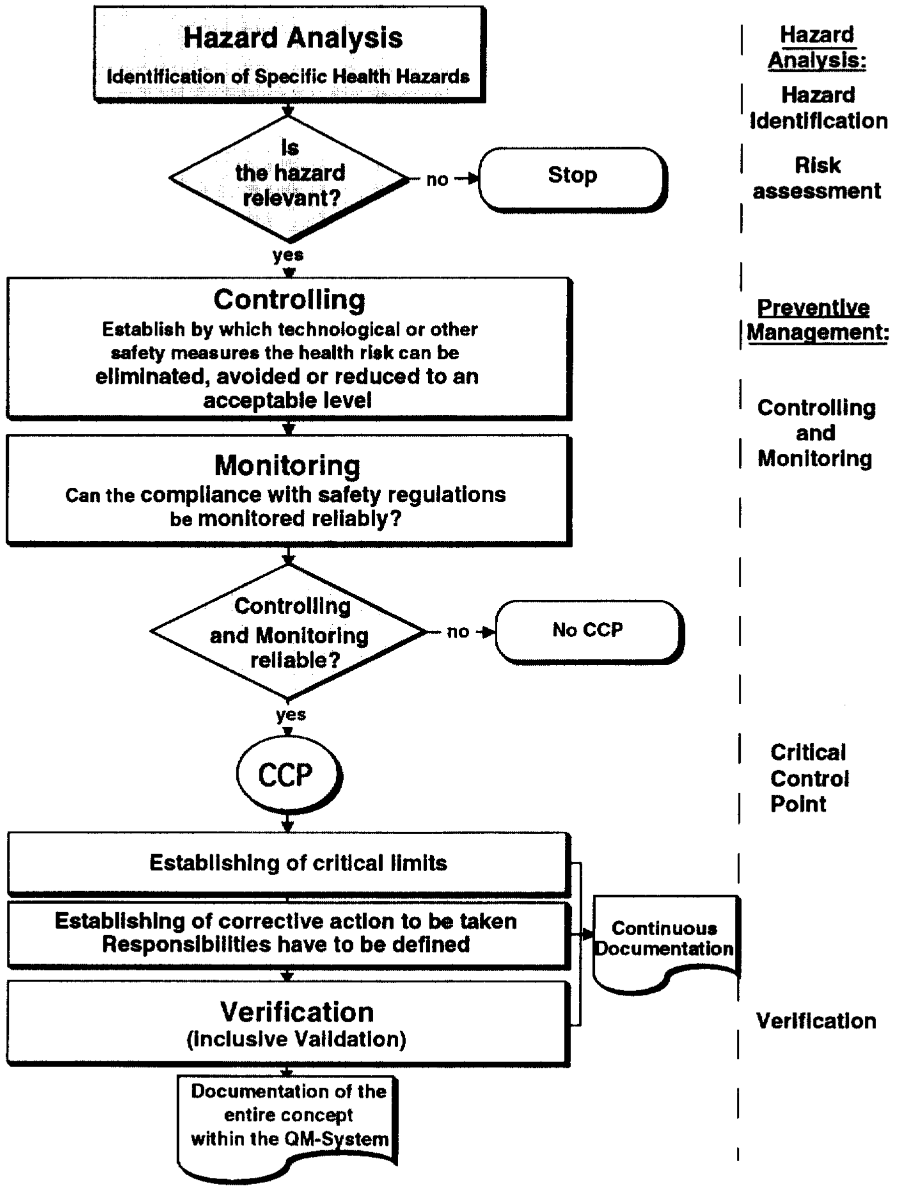

Figure 4 The HACCP concept in food hygiene

context of a HACCP system. Rapid methods can only be applied usefully to the verification of a hygiene assurance concept because they are only suited for the examination of random samples. Reliable monitoring, however, is based on continuous surveillance of the process. According to the FAO/WHO Codex Alimentarius Commission (1996) it is stated that 'if monitoring is not continuous, then the amount or frequency of monitoring must be sufficient to guarantee the CCP is in control'. Unfortunately, the vital distinction between monitoring and verification has not always been made in the literature.

ATP-bioluminescence is an example of a rapid method which, by mistake, has been recommended as a monitoring technique. ATP can be found in microorganisms as well as in somatic cells of plants and animals. Although somatic ATP may be mostly eliminated by certain examination methods it is impossible to distinguish between saprophytes and pathogenic microorganisms. Hence, microbiological hazards cannot be identified with the bioluminescence technique.

THE SIGNIFICANCE OF BASIC HYGIENE MEASURES

With many of the above mentioned types of microorganisms the faeco–oral transmission is of great epidemiological significance. Foods can act as vectors in this process. Food contamination can occur directly via man or indirectly via contaminated water and additionally to some extent by other routes, for example, insects. This is the case with all mentioned virus types and, except *Sarcocystis* and *Toxoplasma gondii*, also with protozoa. However, *T. gondii* is not only transmitted through the meat of infected animals but also via the faeco–oral route from cat to man.

The same way of transmission applies to bacteria, in particular to *S. typhi*, *S. paratyphi*, *V. cholerae* and *Shigella* spp. *C. jejuni* is mainly excreted by human carriers and chickens which harbour the germs in their intestines. As with all pathogens with a low MID the faeco–oral route of transmission from man to man plays an essential role in the spread of outbreaks of EHEC. Hence, foods are mainly contaminated by human carriers.

Considering the particular significance of the faeco–oral transmission route, personnel hygiene constitutes a significant preventive measure alongside the use of good drinking water. Thorough cleaning and disinfection of hands is essential after each use of the lavatory. Food items which are ready to consume must under no circumstances be touched with bare hands. Those who are familiar with the conditions that still prevail in shops, canteens and restaurants are aware of the fact that, in practice, this requirement is far from being fulfilled. Before compliance with these basic hygiene requirements is not assured it does not make sense to initiate the planning of HACCP concepts.

The HACCP system should be seen as part of an efficient total hygiene concept in food companies (Untermann and Dura, 1996). The hygiene concept of a company can be compared to a house (*Figure 5*). The 'foundations' of the 'house of hygiene' are the conditions of premises and equipment. Its 'walls' are the well known basic hygiene measures. They include cleaning, disinfection and pest control as well as temperatures and relative humidity of production and storage sites. Further components are a sufficient separation of production steps and production lines to avoid cross-contaminations and, finally, personnel

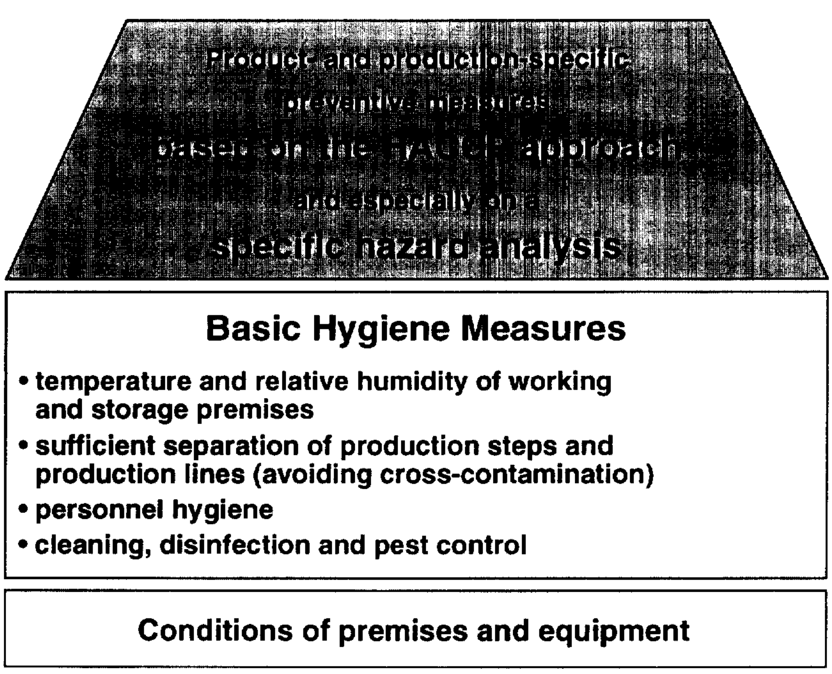

Figure 5 Hygiene concept for food processing companies

hygiene. The 'roof' of the house is made up of product- and production-specific measures according to the HACCP principles to avoid specific health hazards for the consumer.

RENAISSANCE OF OLD PRINCIPLES OF NATURAL SCIENCE

The principles of the HACCP concept are not new. They have already been applied for more than half a century (Untermann, 1996). A very good example for their application is the pasteurization of drinking milk (*Figure 6*) which had already been postulated a hundred years ago as an effective measure to protect man against zoonoses like brucellosis and tuberculosis. Around 1930, this procedure was introduced into German and Swiss food legislation.

In children the transmission of bovine tuberculosis via drinking milk led to an additional important spread of tuberculosis. The transmission of brucellosis by drinking milk was also a significant hazard for consumers. Heat treatment of milk was chosen as an effective control measure which, in contrast to boiling, inactivates the pathogenic bacteria but hardly influences taste and vitamin content of milk. Monitoring is assured by an ongoing temperature control with an automatic writer. Temperatures and times are

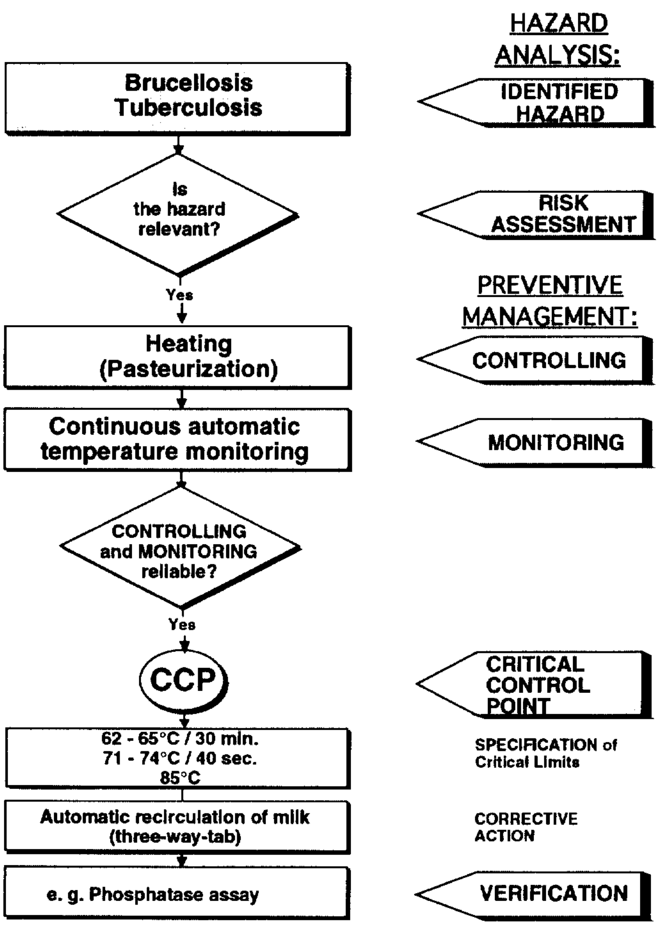

Figure 6 Preventive management in drinking milk production > 60 years ago: exactly corresponding to the modern HACCP concept

defined exactly. Pasteurization apparatuses are thus constructed to ensure that if minimal values are not reached the insufficiently heated milk is directed back into the raw milk storage tank via an automatic three-way tab. Proper heating of commercial drinking milk can be proven by detecting alkaline phosphatase which is normally found in milk and which is inactivated with these heating procedures. This assay is still in use and constitutes a verification.

Hence, the introduction of the HACCP system does not constitute a fundamentally new development. It could even be viewed as a renaissance of old scientific principles.

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