RASFF, HACCP and Risk Assessment in Food Control

Methodological proposal for RASFF, HACCP and Risk Assessment implementation in food control

Exchange of experience and competence between Italian and Slovak Republic food control authorities

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FIRST CHAPTER

INTRODUCTION

White book (2000) about food safety in EU, published by European Committee, is concerned with a thorough revaluation of legislation about foodstuff in countries of European Union. The purpose of it was to create independent European authority for food safety, which first and foremost would have task of foodstuff risk assessment, and to spread existing food alert system on feed, also to increase its affectivity and its spread on products coming from third countries. Spread of system on feed in this way corresponded to general principles, according to which food safety has to be realized according to “from farm to fork” rule. This system as well corresponded to further general rule present in White book which is tracing of food products.

1.1 RASFF

Realization of principles stated in White book was done with regulation 178/2002 EU. In reality this regulation ranks among next important operative and programmable directives, it proves formation of “rapid alert system for foodstuff and feed” (RASFF). In articles 50, 51 and 52 of regulation 178/2002 EU there are determined ways according to which the new system should work.

This system, that is responsible for necessity to inform about every serious risk for public health that comes from foodstuff, feed or its ingredients, helps out with determination and elimination of that product from market. It is organized in the form of net, in which centrals are national contact points, situated in all membership states, and also European Committee itself.

Activation of RASFF for every danger and risk connected with it in its own way presents one stage of risk management.

In Italy national contact point is Office VI of General management of veterinary and nutritious Public Health Service of Ministry of Health Service. This Office receives by e-mail notifications coming from Border Inspection points, from veterinary offices and from offices of sea and air health service. Then these structures directly produce data, that is sent further or gain information from regional services, from veterinary services or information about food hygiene of Local sanitary organizations, from Research zoo

prophylactic institutions or from other laboratories of analysis (Regional agencies for life environment or Multipoint preventive presidia). The consistent groups of products, which were spread throughout Italian market and which were similar to those ones that were notified in case if they come from third countries (consistent five, if a product comes from membership states of EU), are subject to risk control of the same type.

The flow of information is opposite, that is from European Committee for national contact point to periphery in cases where the risk is a product found in different membership state.

The mechanism of fast communication, which is continually more frequent in last years, is an important tool for assessment of possible risk and consumer protection.

The flow of “alert” cases has to provide completeness of information and punctuality of communication.

Notifications in membership states are being communicated and participated with the help of net in real time.

Activity of alert system assumes elimination of products dangerous for human and animal health from market. In case of direct serious risk, besides immediate detaining of products, performed by competent authorities, the situation could be integrated with the help of media.

In this case population is being informed about risk connected with use of certain product and about ways of handing over the foodstuff to local competent authorities, to which that certain region belongs.

In case of products that do not belong to European market and do not need to undergo specific procedures, plain notification will be published from membership states that will help to inform customers about dangerous products and about operated actions, even though necessary interventions will not be realized on the side of receivers of the report.

European Committee reserved space on its own website for on-line consultation of week’s notifications, received from membership states.

On that website it is possible to find week’s notifications divided on:

new alert notification for risk products that are on European market;

new information notification for products that do not belong to European market or are subordinated to certain control actions events by certain country.

1.2 NEW FOODSTUFF LEGISLATION FROM EU MEMBERS – HYGIENIC

REGULATIONS

Hygienic regulations contain general rules in sphere of hygiene for all foodstuff products and for all operators working in food sphere.

It is presented by two hygiene regulations and two regulations about official controls:

Hygiene regulations are designed for producers and regulations about official controls are for competent authorities.

Regulations come into force January 1, 2006.

1.2.1 Reg. 852/2004 EU of European Parliament and Council concerning hygiene of foodstuff products.

This regulation applies to all products and all stages of production and distribution. For the first time in legislation of European Union breeders (basic industry), farmers are considered to be food producers.

Regulation contains principles related to rule system and control “from farm to fork”, according to which the most effective food control system is not any more aimed on products, but on process of production, taking into consideration all stages that create this certain process.

Producer is given primary responsibility in sphere of food safety. In reality producer has to produce goods, taking into consideration the plan of automatic control, that would be prepared and that should regulate all activities for hygienic food production. From all producers it would be required to work in self control regime; this regime would be applied according to rules of Hazard Analysis Critical Control Point (HACCP) for all producers besides those that work in basic industry: breeders and farmers have to preserve “good hygiene practice”.

The HACCP rules state stages that have to be kept for determination and managing of critical control points. All control stages should be recorded in order to prove real application of rules.

Next introduced criterion by this regulation is an obligation for manufacturing companies to have authority or at least to be registered in competent authority; registration is mandatory for everyone, but on the opposite, authorization is demanded only from some types of production and is connected with certain processes of official control.

Apart from what was said above producers are obliged to organize tracing of own products; its goal is to continually be able to eliminate product from market which is considered to be dangerous or also to display the nature of ingredients from which the product is compounded.

Technical supplements deal with general hypothesis for food production and with specific cases of foodstuff of animal and vegetable origin in all stages.

Besides that, they arrange requirements for rooms, conditions of transportation, waste, and access of water, hygiene of staff, packaging, processing and training.

Products imported from countries that are not part of EU have to be in agreement with requirements given by that regulation.

This regulation abolishes directive 93/43.

1.2.2 Reg. 853/2004 EU of European Parliament and Council, which regulates specific directives in hygiene sphere of animal origin foodstuff.

The sphere of application takes into consideration all animal origin foodstuff products, but special attention is put on fresh meat (red meat, poultry, and venison), living mollusks, fish, fresh milk and milk products. It contains a determined task of official veterinary surgeon in sphere of production of raw red meat, who realizes the activity of official controls divided on controls in form of auditing about processes of self control and inspections about information about processing chain, visits before and after animal death (ante a post mortem), materials with specific risks, laboratory tests, medical branding. It also defines specific activities of inspection staff in relation to training needs necessary to gain qualification and its upkeep.

1.2.3 Reg.183/2005 EU of European Parliament and Council, which determines hygienic requirements for feed.

The responsibility concerning producers of feed is equal to the one that relates to producers of foodstuff.

Companies working in that sphere are obliged to register in competent authorities and self control is being realized according to the rule of “good hygienic practice”.

Regulation contains specific hypothesis of good practice of animal feeding during grazing, in cowshed, and supply of water.

1.2.4 Reg. 854/2004 EU of European Parliament and Council, which determines norms of organization of official controls for foodstuff products of animal origin that are designed for human consummation.

This regulation applies to foodstuff products of animal origin and contains seventeen current vertical directives. It does not apply to stages of retail distribution and to compound products.
Processing companies of these products must undergo authorization of setting an identified number given by competent organs.

Alongside foodstuff chain there should be mandatory exchange of information.

The regulation describes requirements for production of home meat, poultry, bred and hunted venison, minced meat, meat preparation, mechanically separated meat, meat products, living mollusks, fish, milk and milk products, eggs and other various products of animal origin.

Technical supplements contain requirements for tools, rooms, marking and branding, hygienic requirements, temperature standards and microbiological criteria for different types of products.

1.2.5 Reg. 882/2004 of European Parliament and Council concerning official controls, that are operated for verification of concurrence with regulations in sphere of feed and foodstuff and verification of concurrence with regulation about norms of health and comfort of animals.

The regulation helps official controls of foodstuff products made for human consummation to be more effective and wants to help membership states with verification in consideration to national and European enactment in sphere of foodstuff legislation.

Seven vertical directives are abolished.

According to this regulation official controls have to be on the basis of regularity, without notification in advance and in whatever stage of production or distribution of foodstuff or feed. They have to be based on the basis of risk analysis, on already obtained experience, and reliability of controls realized by that same operators.

Membership states name competent organs for performing of official controls. These organs have to work according to principles of impartiality, affectivity and concurrence.

Besides that, suitable conditions and specially trained and qualified staff should be available. Correct coordination between prospective central and peripheral components of system is necessary.

Some functions, as for instance activities concerning auditing, inspection and laboratories, can be delegated to organizations which do not belong to public sector, but these organizations have to be subject to valuation by membership states.

Membership states must have worked out many years' control plans that they will present to European Committee:

•structure, organization, determination of control authorities

•goals and sources

•possible mandates (assessment ways)

•plans for crucial situations

•training of staff

1.3 LEGISLATION IN PREPARATION STAGE

The preparatory legislation should be taken into consideration in sphere of foodstuff safety:

1.3.1 Regulation about flexibility of HACCP application in small companies

Proposal of regulation assumes observation of what is given in Regulation 852/2004 paragraph 15 article 5, criteria to determine types of manufacturing companies, in which level of production explains simplified application of self control according to HACCP rules. It concerns companies that either because of the production level or type of products guarantee control of risks with the help of the simplest methods of HACCP. In this cases proposal assumes flexible way of HACCP application in certain foodstuff companies, reducing self control to implementation of good hygienic practice, in different words to assumption of HACCP itself.

1.3.2 Regulation about microbiological criteria

Proposal of regulation assumes determination of critical limits for some risks and for certain types of products that have to be taken into consideration by processing operations during application of self control according to HACCP rules.

1.4 OFFICIAL FOOD CONTROLS IN ITALY

In Italy the Ministry of Health Service is responsible for official controls. It works as the competent central authority with the help of “Management of animal and foodstuff health”. This authority has nationwide competence in sphere of national and international legislation, in sphere of auditing, in consideration to regional authorities, inspections in border points and in all areas concerning safety of foodstuff and veterinary sector.

On a regional level in every region there are departments of health service that fulfill function of contact point between Central authority and Regional authorities. Regional authorities are represented by departments of prevention of local health service enterprises (approximately 160 on a district level covering whole national territory). In every prevention department there are services of foodstuff and nutrition hygiene (SIAN) and veterinary services (SVET).

SIAN is competent in nutritious sphere and in sphere of foodstuff hygiene in general; SVET is competent in sphere of health and comfort of animals, feed and official control of all foodstuff chains of animal origin with the help of its functional areas: business health service, hygiene of zoo technical production, hygiene of animal origin foodstuff.

SIAN and SVET have authority and inspection functions, auditing functions for self control, sampling in all enterprises which directly or indirectly intervene in basic industry, transformation or distribution of foodstuff and feed.

Staff that works with actualized program is trained in medical, veterinary, technical and administrative spheres.

Activities of health controls at the borders, such as inspection border points (veterinary surgeons) and Offices of sea and air health (doctors) directly depend on Ministry of Health Service.

Official laboratories are represented by Zoo prophylactic Research Institutes (IZS) with 10 central residences and 90 district residences for products of animal origin and feed and regional agencies for protection of animal environment (ARPA) with 20 central residences and 90 district residences for other foodstuff. All the laboratories are accredited by norm ISO17025.

Mentioned above centers also realize activities of epidemiological observation and collection of data for territories with its competence, research and training (in cooperation with universities).

Risk assessment is provided by research centers IZS and ARPA, universities, Supreme Health Institute and National Council for foodstuff safety.

1.5 INFORMATION FLOWS

Results of inspection and laboratory activity are transferred from periphery units to regional and from there to central database.

Local doctors and hospitals are obliged to fill local flow of information found or assumed pathology connected with food. Epidemiological searches are being made on the basis of these indications in order to find out reasons of that certain case, taking into consideration SIAN and SVET according to the type of competence.

Results of the searches are being sent to national database from where the data is taken to be used for foodstuff risk assessment.

1.6 RASFF IN ITALY

1.6.1 Alert at the entrance

Information is being delivered by e-mail reports.

Information is being delivered from European Committee from the Office of foodstuff safety and distribution chains to the Ministry of Health Service.

The Ministry of Health Service informs veterinary services and foodstuff hygiene and nutrition of involved regions.

The regional services process entry information for district SVET in case of products of animal type or for district SIAN in case of other types of products.

The notification will get to:

- district official in charge of alert management for following activities of SVET or SIAN;

- responsible individual of SVET or SIAN.

District official in charge of management of alert cases activates all functionaries of the regions with products that are reason of alert.

Local functionaries: in relation to distribution of mentioned above products local functionaries will implement controls and take urgent steps to limit the expansion of danger. In areas where the situation is crucial they will work with technical research aid of IZS and ARPA and operative help of regional services for managing crisis situations.

Consequential and final steps will be made by local functionary on the basis of sending a responsible person from district SVET or SIAN.

District official in charge of managing alert cases and notifications takes care about realizing of steps given by alert system and if it is necessary coordinates connections with other further local units that are possibly involved in the crisis.

In case of refusal of products (in relation to supplier) that are subject to warning or notification and in case if given products were distributed to other geographical areas, district official gives local functionary necessary aid for notifications. The refusals are to be sent to other inspection offices.

District official directs a document of warning or simple notifications in adequate archives that would contain notification, documents implemented by inspectors, notifications sent to other offices, regions, to the Ministry of Health Service and taken steps.

He will manage and maintain actualized electronic archives of alerts and plain notifications.

Regularly by e-mail he will announce results of implemented controls and taken steps to responsible district individual from SVET or SIAN.

Responsible district individual from SVET or SIAN verifies and cancels notifications proposed by district official in charge who sends it to the Region or to the Ministry of Health Service.

1.6.2 Alert at the ascent

In case if local functionary of SVET or SIAN finds out the presence of direct and indirect risk for human health, he immediately delivers that information to district official in charge of managing alert cases and to district responsible individual of SVET or SIAN.

Local functionary takes urgent steps necessary to limit the spread of danger. In areas where the situation is crucial he will work with the help of technical research aid of IZS and ARPA and operative help of regional services.

Consequential and final steps will be made by local functionary on the basis of directing a responsible district person from SVET or SIAN. Every taken step has to aim at restrict market distribution or to strengthen elimination of food and feed from market.

District official in charge of alert cases announces to the Region taken steps and besides that all known information that concerns the level of risk.

Consequentially the Region sends information to the National contact point for alert cases.

Consequentially RASFF is activated.

In case if this information comes from Border control point the information is being sent directly to the National control point for alert cases. In that case a responsible individual from control border authority takes urgent lasting steps according to the analogous procedures of district offices.

SELF CONTROL ACCORDING TO HACCP METHOD

SECOND CHAPTER

INTRODUCTION

HACCP method is a preventive system made for providing *medical safety* of foodstuff with the help of identification of specific risks, its assessment and preventive control and managing activities.

That new philosophy founded on objective and quantifiable components is combined with direct responsibility of producer and in this way creates complex hygiene-medical consuming system with new preventive access.

This proposal appeared from experience connected with implementation of HACCP method in various foodstuff manufacturing companies (beginning with bakery, through vegetable cans, sector of smoked products, and up to restaurant settings) and aims at simplifying of logical consequence of components necessary for elaboration of manual.

Used method is based on distinction of real productive experience that is in whatever way regarded as an indispensable requirement of true HACCP method which divides it into two parts: the first concerns requirements and the later HACCP development in the production line itself according to schema.

2.1 PRELIMINARY ACTIVITIES FOR IMPLEMENTATION OF THE PLAN

Before activation of working programs for implementation of the plan it is necessary to include all professional individuals, who work in company, for general training about HACCP and consequentially for specific training program for those individuals who perform key tasks.
All is being activated from the level of enterprising management (mandates from the board of directors) who by their competence actively interest operators with the help of split of functions and responsibility and besides that provides sources and tools.

Warning: Some models of control schemas, programs, registers, etc. are represented in contemporary contents. These examples will be used as foundation for processing specific to enterprising documents.

2.2 DESCRIPTION OF CONTENTS

According to specific criteria requirements are to be written down first in self control plan, and production as the following. The table shows the example of parts that necessarily should be present in manual.

CHAPTER 1 PRELIMINARY REQUIREMENTS	CHAPTER 2 PRODUCTION
ENTERPRISING DATA	DEFINÍTION AND DESCRIPTION OF PRODUCTS OR PRODUCTIVE LINES
MANDÁTE MANAGEMENT	IDENTIFICATION OF DESTINATION GROUPS
GOAL OF THE PLAN	DESCRIPTION OF PRODUCTION PROCESS
HACCP GROUP	DIAGRAM OF PROGRESS
LOCALITY AND EQUIPMENTS	VERIFICATION OF DIAGRAM OF PROGRESS
SANITÁTION	RISK ANALÝSIS
UNDESIRABLE ANIMALS (PESTS)	INDIVIDUALIZÁTION OF CCP
STAFF TRAINING	CRITICAL LIMITS
STAFF HYGIENE	MONITORING
HYGIENE IN WORK	CORREČTING ACTIVITIES
REGULAR AND SPECIAL MAINTENANCE	VERIFICATION
WASTE PROCESSING	DOCUMENTÁTION
CONTROL OF WATER	SAMPLING
TRACING	

LABELING	
RECEIVING AND STORAGE OF INGREDIENTS	
RECEIVING AND STORAGE OF MATERIÁL FOR PREPACKAGING AND PACKAGING	
RECEIVING AND STORAGE OF RAW MATERIALS	
QUALIFICATION OF SUPPLIERS	

In the **first chapter** concept of SVP "requirements" (Good Manufacturing Practice, GMP) defines everything that company has to implement in order to make optimal conditions for work. Those kind of procedures have universal validity, are centered on structures, equipment and staff, in practice they have "transverse effect" – procedures go through production, controlling sources of contamination, usually do not design monitoring planned with appropriate registrations.

Part of procedure "requirements" are SOP (Standard Operating Procedure) which intervene with manufacturing process helping to reach ideal conditions of animal environment for foodstuff production. They serve as control of specific risks and guarantee its control/management, especially on the level of risks that are hardly manageable on the level of separate production stages. Procedures serve as control of operative conditions in enterprise including operative instructions for control/management of process parameters.

With the help of SOP already controlled and managed risks would not have to be taken into consideration during risk analysis, and in this way would simplify HACCP plan.

Procedures have to be documented, applied and monitored systematically.

To choose managing SOP procedure or GMP in enterprise is a function of risk level of food production.

Procedures that can be managed in SOP are:

a. **Sanitation**
b. **Pests**
c. **Control of drinking water**
d. **Waste control**
e. **Maintenance of instruments and equipments**
f. **Hygienic and medical controls of staff**
g. **Choice and verification of producers**

Every separate procedure has to contain:

1. **goal and field of application;**
2. **responsible individual with representative;**
3. **enterprising conditions;**
4. **documentation.**

Enterprising conditions will be illustrated according to the following criteria:

a. **Program with frequency**
b. **Conditions**
c. **Monitoring with limits**
d. **Corrective activities**
e. **Verification**

In case if procedures are applied to external staff or external enterprises, a responsible individual, besides processing all documentation, performs periodical verifications in agreement with settled operational protocol.

In the **second chapter** attention is paid to production and application of 7 rules of HACCP for every product or production line on the level of separate stages according to the following order:
Lead: *analysis of risks*
Identify: c*ritical control points* (CCP)
Determine: *critical limits*
Define: *monitoring procedures*
Define: *corrective activities*
Define: *documentation*
Determine: *verifications*

It comes true by means of application of intervention plan intended on prevention of foodstuff risks by connecting with already available rules and methods such as statistical analysis of results, archives of documents, qualification of suppliers, control of process and etc.
Besides preventive definition of specific corrective activities for maintenance of production process under control, the program named HACCP embraces risk identification over food production, its arrangement according to hierarchy, acceptance of specific preventive steps for risk control/management, definition of critical control points, which are stages of process, on the level of which control is effective and economical.

In order for application system HACCP to be effective and purpose-build, **mandate from the board of directors** is central part of the system, without which hygiene-medical goal would be neither shared nor respected.

If accurate mandate is absent, first people that will be affected are workers who are authorized to work

according to habits and customs, not maintaining operative instructions motivated on the research base. Consequentially, in practice it would be manifested that money and sources were invested to fulfilling norms, but without practical results.

It is also caused by insufficient knowledge of basic concepts of HACCP, its transfer to bureaucratic system that does not lead to reaching of particular goals and from the side of management does not share the aspect of safety.

Next aspect of primary meaning is that external advisors are absolutely authorized. It concerns homogenization of manuals of self-controls which look the same from the point of view of production type and do not take productive reality into consideration. It leads to superficial risk analysis without appropriate competence, what brings it to the uniformity of risk according to the type of production without taking into consideration the history of company or implementation of appropriate preventive steps of managing, for instance absent preliminary proper actions, which would keep the requirement of minimal proper actions. Monitoring system was interpreted as the system of verification, corrective activities are absent, whereupon it comes out of assumption that those do not get confused, documentation is insufficient and is not processed according to objective criteria, what brings it to the entire loss of preventive goal.
During development of quantitative type HACCP details preventive system, whereupon concentrates economic and instrumental instrument sources where it is necessary.
Next difficulty on the side of producers is tolerance of time need, because preventive system gives results after a long time.
On the basis of all experience there is a need to define application of concomitant lines, which during the application of the Code rules determines those ones that are minimal proper procedures that is necessary to maintain throughout self-control plan.

2.3 REQUIREMENTS

2.3.1 Corporate data

Define:

a. Typology and quantity of enterprising production
b. Possible seasonality and/or periodicity of work
c. Add enterprising organization plan where there are enterprising functions and responsibilities which can be summarized in a table.

2.3.2 Mandate of directory

The success of HACCP plan depends on active joining of management.

Management should give support and necessary sources (finances, staff and time) for development and application of plan. In artisan's enterprises it is enough if owner, who is convinced about function of system, transfers input on co-workers.

2.3.3 Goal of the plan

It defines operational stages for realization of the plan, and division into stages beginning with attentive valuation of preliminary requirements.

Only when the preliminary requirements are valuated and implemented, the dispensation of HACCP plan can start.

Operational plan that proceeds with the help of models will be being prepared in practice. In this way preliminary requirements will be connected with HACCP.

2.3.4 HACCP Group

The manual of self control is written by company cooperating with many individuals. Information about staff involved in production is being checked.

2.3.5 Rooms and equipment

***Rooms that are subject to identification are*:**

Outside rooms and space for storage or processing of secondary products and waste
Rooms for loading and unloading
Corridors and transport routes
Storage rooms
Working rooms
Places of selling
Service places (lockers, accessories)

Rooms that are subject to special identification are:

Distribution, storage and quality of drinking water
Steam production
Processing of waste
Additional cooling
Electric equipment and mains

All working instruments and equipments

All pluviometers belonging to enterprise are given in supplement of the manual (pluviometer of institution, lay-out of equipment and staff, pluviometer of unloading, pluviometer of water circulation with evidence and numbers of mains points, plane metric, places of hydrants…).

Documentation proving identifying number CE or competent authorized medical documentation should be enclosed.
Those kinds of authorizations guarantee suitability of structural characteristics and institutions. In any case description of structures and its organizations is recommended.
Installations, instruments and equipments must be designed, constructed and installed in order to avoid risk connected with product.

Materials
All materials used in enterprise have to be for foodstuff use and have ability to resist mechanical damage, chemical corrosion and biological decay.

Monitoring of environment
It would be operated regularly by visual control of hygiene medical conditions of rooms, equipments and instruments, or always when modifications are being applied, or when special maintenance is being operated regularly or with specific meaning.

2.3.6 Restaurant sector

Restaurant sector contains rooms, appropriate installations and equipment, for instance storage of products that easily and hardly decay, accessories, storage of waste, dish washer, kitchen and etc. (accurate identification enables description of manufacturing process, diagram of progress and placing of control points throughout the whole process).

Example:
Applicable equipment and installations:
Refrigerator, pots for cooking pasta, ventilation pipe and etc.

Fields of cooking:
(“everything in advance” principle) easily washable, without dead corners well identified in plane metric

1 preparation of meat, poultry and fish;
2 vegetables;
3 cold meals;

4 cooking;
5 portioning and prepackaging;
6 serving;
7 special diets;
8 confectionery;
9 delivery self-service;
10 washing of pots;
11 washing of plates;
12 hygienic services;
13 storage of waste.

2.3.7 Sanitation

Sanitation is a foundation of good manufacturing practice and is presented into two stages: cleaning and disinfection.

This procedure can be completely or partly settled with external enterprises. In that case it is necessary to enclose contract with possible verifications.

Procedure is defined for regular and special operations. Regular operations concern certain principles according to which they are operated. Special operations concern accidental phenomenon, such as fall on the ground, instrument contamination, intervention during work or break, unfavorable results of monitoring and verification, besides phenomenon a plan of corrective operations should be defined.

Every procedure has to contain:

1. **goal and field of application;**
2. **responsible individual with representative;**
3. **enterprising conditions;**
4. **documentation.**

Enterprising conditions will be illustrated according to the following criteria:

a. **Program with frequency**
b. **Conditions**
c. **Monitoring with limits**
d. **Corrective activities**
e. **Verifications**

Sanitation plan is expressed in the following points:

Regularity - program

Enclose structures and equipment for every working place, marking operation of cleaning and disinfection, product being used and appropriate active principle for each frequency.

Techniques:

Define how to perform sanitation and with what equipment, what chemical products are used and how (concentration, periods of contact, dispensation of pre-operational and operational activities, temperature being used and conditions of dilution). It is necessary to make TECHNICAL TABLES of chemical materials used in operation which present all the information necessary for correct use. In case if the products are bought directly from retail, it is often hard to find technical tables. In this situation it is enough to enclose later label of product with main information about use such as conditions of dilution, periods of contact and concentration of active principle. Storage of sanitation accessories is usually found in planimetry.

Program and conditions for sanitation can be summarized in one table, specifying list of products (at least 2 for change) for every stage of cleaning and disinfection, and defining calendar for regular cleaning.

Table of program and conditions of sanitation, ex..:

Equipment/ structures	**Products** (cleaning supplies, disinfection)	**Concentration of use**	**Conditions of use**	**Frequency**

Table of regular cleaning (excluding daily cleaning), ex.:

Week - date	**Weekly cleaning**		**Cleaning once in 15 days**		**Cleaning once a month**	
1/1/00	…….	X	…….	X	…….	X
8/1/00	…….	X	…….		…….	
15/1/00		X		X		
22/1/00		X				
29/1/00		X		X		

Monitoring

Responsible worker controls state of cleaning in pre-operational and operational stages through sensory inspection verifications and complete the “table of sanitation control” (check-list).

In divided and complex manufacturing structure it is necessary to regularly fill the CONTROL TABLE. In small structures the registration is being made with consideration to the sources disposed.

Procedure is being verified when after operation and assessment it reaches hygiene-medical goals.

Besides control-monitoring results, corrective steps taken in case of accidents are also being recorded.

Example of control schema of sanitation:

Week from _____ to _____ person responsible for cleaning _____

	Agreement	Disagreement
Pavements		
Walls		
Working tables		
Cleaning bathroom		
Other (define)		
Total		

note
in accordance: everything is perfectly clean
in discord: dirt on surfaces in contact with food

CORRECTIVE ACTIVITIES:
ANY disagreements assume second additional sanitation before beginning work.

Possible disagreements because of insufficient following of program or repeated mistakes demand verification or revision of sanitation program.
In every case all facts which are in disagreement with appropriate corrective activities will be recorded in the index of discord.

Assessment of responsible person on the basis of sensory analysis, taking into consideration the following parameters:

2 absence of unusual smell or colors
3 absence of visual dirt
4 resistance to scratch with fingers
5 white napkin test
6 water drop test
7 dry surfaces
8 operative conditions of employees during application of protocol

Verifications

The program for sampling of regular and special microbiological control of surfaces (microbiological or bioluminescent).

Microbiological tests will be planned (frequency is in accordance with manufacturing typology) for all structures and institutions; mentioned tests are based on natural drosses (cotton tampons, tampons from algal material, contact dishes, washing) and are operated by appropriate laboratory staff or staff from enterprise itself.

Places of taking, instruments being used, sampling conditions, analytic methods, frequency, limits of acceptance and corrective activities are recorded as well.

Microbiological disagreements assume acceptance of corrective steps: sanitation and repeated taking of drosses from surfaces. Those kinds of disagreements will be recorded in the index of disagreement.

Good practice assumes record of all data with date of contact and all necessary information about procedure.

Example of corrective activity for unfavorable microbiological result: special sanitation, intensive control after three results in a row, which would be in agreement, and then return to regular verification plan.

Documentation

Actualization of procedure is being represented with the help of the following documentation:

- Responsible person
- Plano metric
- Protocol of preoperational and operational cleaning, including verification plans
- Technical tables of products
- Activity plan where special intervention will be necessary
- Specific staff training
- Registration of control-monitoring intervention and verification of appropriate results
- Registration of corrective steps

2.3.8 Undesirable animals(pests)

Except managing of possible chemical materials it is necessary to state protocol of intervention in relation to undesirable animals (dogs and cats) and infectious animals (rodents, insects and etc.) that can be a source of direct or indirect infection of food.

It is then necessary to plan intervention or to document results with the help of monitoring plan for assessment of intervention affectivity and appropriate corrective activities.

For definition of these procedures it is suitable to define preventive plans and interventions (with consideration to daily hygiene of environment and equipment, regular garbage disposal, protective equipment, closed and protected doors and windows, inside and outside maintenance of buildings,

equipment settled without left space, control of animal rests from the side of staff).

Interventions are being operated by internal staff of company or specialized external enterprise. In latter case operative control should be enclosed in manual and has to be regularly verified.
It is suitable to have program of intervention in advance, defining frequency, conditions of intervention, list of products used in program with appropriate technical tables and table of evidence.
It is necessary to enclose the plane metric of room enclosing baits.

2.3.9 Staff training

Staff of enterprise will visit trainings concerning good practice of operating and manipulation with foodstuff products. Staff was in that time informed and recorded to working on the manual of automatic control.
Besides that it is suitable to define programs of technical-research education, which is multilevel in relation to experience, tasks and level of education of staff.

Items stated in definition of formation program:

1 contents,
2 initial and final criteria of assessment of acquiring knowledge,
3 informative method (theoretical, theoretic-practical, practice).

A manual usually contains collection of certificates of participation. Affectivity of training is being valuated at working place checking habits and behavior of staff. Possible lacks are to be subject to actualization of separate training.

2.3.10 Hygiene of staff

The regulation states the rules that staff is obliged to follow, and principles that help in training, such as:

- Operators wear working uniforms, shirts and pants of bright color, clean and without spots or fat and pockets, suitable shoes, easily washable and disinfected;
- Frequency of uniform changing;
- Ways of washing uniform with specifying people who do this work;
- Working uniform is used solely inside of company;
- Operators wash their hands often, but especially before entrance into working rooms, after all breaks and after manipulation with raw materials, semimanufactured products, end products in order to avoid cross contamination;
- Working uniform should cover all clothes;

- Wearing a net on head that covers all hair is mandatory;
- It is forbidden to smoke, eat, wear all kinds of necklaces or other things in working environment;
- Possible cuts, wounds, scratches, burns or infections have to be immediately announced to responsible person and in every situation before beginning of work be well protected;
- Preferentially is to use gloves for one time use;
- Gloves for more uses after cleansing from grease have to be washed with cleaning supply and disinfected in boiled water after every use;
- Protective metal gloves after cleansing have to be disinfected in warm water at the temperature of 82°C, at least after every longer break;
- Use of masks is indispensable at working places where there is danger of contamination or re-contamination by products that are less taken care of (ex. packaging room);
- Every wash basin has a tap (not manual) with warm and cold water, with liquid soup, disinfection material and garbage can that opens with the help of leg.

Enterprise determines one responsible person for verification of good application of enterprising procedures in field of staff hygiene. Besides that it states:

1 Discipline of arrival at working places
2 Typology of working clothes including ways of cleaning
3 Storage of working clothes

Responsible person constantly controls usual hygienic procedures of staff. In case if the disagreements are found, it will be justified and recorded to the index of disagreements. In real divided and complex enterprises it is necessary to regularly fill the CONTROL TABLE. In small enterprises registration is being operated with the consideration of the means at disposal.

2.3.11 Hygiene in work

Regulation states operative instructions that staff have to follow during work.

Things specified at the first place:

- movement of foodstuff inside the company by suitable means so that it will not come into contact with goods (means for transport must correspond hygienic principles);
- disposal of covers and packets;
- time of non-working intervals between different manufacturing stages and/or productions;

- defrosting;
- freezing;
- cooling during cooking;
- canning in warm environment;
- control of temperature (cells, environment, technological treatment);
- cold production;
- washing of vegetables;
- serving;
- self-service;
- serving in different ways;
- cooling;
- rooms for processing.

2.3.12 Maintenance of instruments and equipments

Program - Frequency

All enterprising structures and equipments that have hygienic-sanitary importance have to be regularly controlled by **regular maintenance** to guarantee good functioning.

Extra maintenance is being operated when there are interventions of extraordinary character (unforeseen) that have to be taken care of in case of every kind of damage. Those kinds of damage will be recorded in the index of DISCORD, with appropriate corrective steps, event, date and case.

This information can also be summarized in tables about separate equipments or in enterprising records dealing with these problems or in control tables.

Monitoring

Enterprising instruments that have hygienic importance are to be recorded in one table, including measuring instruments, as thermometers, pH-meters and weights.

Regular maintenance and regularity of it is to be stated for every separate instrument.

Every time when enterprise applies to external company in order to operate maintenance (regular and/or extra) it records documentation in protocols that contain type of operated intervention and controlled result.

Continuous monitoring of condition of maintenance and use of structures, equipment and instruments help to avoid accidents, wrong operation or false surveys.

Besides program of regular maintenance and definition of procedures of extraordinary maintenance, there can be made a CONTROL TABLE OF RECORDED INSTRUMENTS with dates and operated interventions with instruments measuring parameters, which are subject to control (thermometers of cooling boxes, weights, heaters and so on). Controls will be operated with the help of planned and consequential maintenance and serve for verification of reliability condition of an instrument. This procedure is obligatory for instruments used during CCP monitoring.

Control of equipment for monitoring

It is necessary to define control procedure and calibration for equipment for CCP monitoring, describing detailed control method and ways of use.

Description of control ways has to follow regulating operations, based on instructions added to equipment or based on comparison with exact instruments. It is necessary to define regularity of these operations.

Regularity of control is changeable depending on reached results that are level of displayed reliability.

Control of function of poking thermometer can be done by comparison with corresponding thermometer, used only for that reason, with the help of three measurements in a row with distance of 1' for every instrument, first in boiling distillated water and then in melted ice. Difference between average of three measurements should range between <0,5°C up to <1°C. For higher results it is necessary to adjust thermometer.

Intervals of regularity of calibration have to be shortened in case of not exact instruments, or should be lengthened if results guarantee sufficient reliability of instrument.

The following is an example of control table for recording data:

2.3.13 Waste disposal

Ways of processing of waste should be recorded beginning with:

- type of waste – organic, liquid, gassy, municipal garbage;
- the places where garbage is taken during work;
- places where it is stored before removal from enterprise;
- regularity of removal from working places;
- person who collects garbage (copy of contract);
- possible documentation about future processing;
- ways and regularity of control of emptying;
- ways of collection, canning and processing of secondary products from categories 1, 2, 3. (Reg. 1774/02 EK).

For liquid garbage it is necessary to enclose the plan of rooms where it is stored with marking points of waste stores and its possible removal.
Possible control and analytic records are enclosed in supplement.
For organic waste it is necessary to enclose declaration of future processing. Possible disagreements will be recorded in the index of disagreements.

2.3.14 Drinking water

The use of drinking water, including ice and steam, is obligatory, when it comes to direct contact with not protected foodstuff.
It is necessary to enclose the plan of water main with numbering of outlet points.
If the chlorine device is being used, it should be equipped with visible and loud warning system, so that signals in case of bad function enable immediate intervention, and also automatic blockading of water supply. Rest chlorine is being regularly dosed. After processing and before installing into the net it is necessary to dichloride water in order to avoid presence of rests exceeding allowed measures.

This kind of device should be added into the program of maintenance and person responsible for the procedure should be able to renew function, regulate system of chlorine dosing and filters for chlorine.
]

Water tanks or other systems of water supply must be taken care of, situated in perfect conditions and regularly checked and cleaned, as it is mentioned in the program of maintenance and sanitation.

It is also necessary to settle the program of water control, operated tests and regularity. Regularity of chemical and microbiological analysis is related to the supply source and, as it is mentioned in recommendation EK 89/214, and have to be operated inside of enterprise and at all outlet points.

Possible disagreements are recorded in the index of disagreement, taking good practice as early information for public control authorities.

Drinking water can be used for:

- cooling of instruments;
- steam production if the latter does not come into direct contact with foodstuff;
- fire settings

2.3.15 Tracing

Every enterprise has to process written procedure for tracing with the help of giving number to the certain group of products. The number is given already during selling of foodstuff product, gained in equal technological conditions, during upkeep of way of delivery from enterprise and elimination of products from market, potentially dangerous for customer.

Meaning:

2 delivery when selling of not yet distributed product is excluded;
3 elimination, when it is necessary to settle measures into practice for exclusion of consumption of already being sold products

For enterprise to begin procedure of elimination from the market it is necessary to have actualized list of clients and registration of destination groups for every group of products, including at least name of sale, number of groups of products and manufacturing company.

2.3.16 Labeling

Establish general principles for arrangement of labels; information that has to be enclosed is given (according to the legislation).
Copy of all labels will be enclosed to the manual.
In enterprises with EU identity there should be index of labels being used and eliminated, together with expected EU sign.

2.3.17 Acceptance of food additional materials and other ingredients

It is necessary to fill index of additional materials and other ingredients used in enterprise, adding list of technical tables in the manual, and a list of producers specifying if they are certified.
Besides that stages of sorting ingredients, specifying how (bags, 1 kg packages and etc.), stages of storage (temperature, dry environment and etc.) before use and opening of package should be also recorded.
It is essential to specify which controls will be made with raw materials, what materials, methods and its regularity.
Possible analytic records will be reported in the index of tests and possible DISAGREEMENTS with type of product will be specified in the index of disagreements.

2.3.18 Acceptance of material, prepackaging and packaging

As for additional ingredients it is necessary to prepare:

1 list of used raw materials;
2 list of producers specifying if they are certified;
3 enclose technical tables, proving suitability for food use;
4 description of ways of delivery, operated controls during delivery and storage, specifying where the unpacking takes place before use and enclose technical tables to the manual.

2.3.19 Acceptance and storage of raw materials

Raw materials should come from known and authorized enterprises, must be imported and delivered with consideration of parameters specified by producer; must be in agreement with norms of labeling; must be integrated and not dirty.

It is important to define the producer's ways of production and storage (this information is essential in order to know if there are used any anti parasite materials for canning, for example, plants coming from biological cultivation or from integrated groups of products).

The lists of all materials, including roots and aromas, bought by enterprise are to be enclosed. Every raw material is subject to consideration of important hygienic sanitation aspects, as for instance:

1 definition of product;
2 characteristic (weight, measurements, type);
3 type of prepackaging and packaging;
4 chemical-physical characteristics (pH, $a_{w,}$ concentration of solvents) and biological (appropriate micro flora), in order to control pathogenic microorganisms;
5 possible biological contamination;
6 conditions of canning before use, including preferential time of use;
7 control of sorting;
8 ways of producer's production and storage;
9 set of time for delivery with suppliers so that control of taking over is guaranteed.

For raw materials that do not have reliable data accessible from literature or gained from the experience of operators, the information can be demanded from supplier or additionally verified. In case if the information is not offered, it should be defined directly in enterprise with the help of sampling plan.

Ways

Besides ways of storage in description of conditions for delivery, ways of control and verifications, special attention is paid on:

1 ROMMS, INSTRUMENTS AND EQUIPMENTS

2 STAFF

3 TIME

All corresponding information is enclosed n the “delivery of raw materials” table. If necessary the sampling plan would be defined depending on enterprise and type of supply.

In case of primeval raw materials that are not subject to risk, it is enough to verify agreement with order, indications on labels, possible duration period and undamaging of package.

Monitoring

Raw materials marked as dangerous have to have its ACCEPTANCE TABLES of primeval raw materials that are connected with company progress, being regularly supplied.

It is helpful to make acceptance table for documentation of types of control and especially for corrective interventions.

It is essential to give different information in the table:

- Date of supply;
- supplier;
- basic data of accompanying and/or medical document with special data of identified group of products;
- specification of products (physical condition, typology of product, possible packaging and covers);
- data of person, who operates and person, who oversees information;
- sensory parameters found during inspection (color, smell, presence of visible changes and so on), together with its evaluation of correspondence (corresponding, acceptable, not corresponding);
- chemical and physical findings (pH, temperature…);
- possible taking of samples for microbiological analysis (photocopy of findings have to be enclosed in the table);
- corrective interventions necessary in case of disagreement with acceptable parameters (consideration is taken to disagreement with parameter), warning from supplier, rejection of goods.

It is considered necessary to present meaning of words and describe needed corrective interventions in the table in case of DISCORD.

Possibly found DISCORDS have to be enclosed with its appropriate corrective intervention into the index of DISCORD.

After filling the tables are kept as supplements.

In case if microbiological analyses of raw materials are done at the entrance, it is needed to add this information to the table.

Storage of raw material

It is necessary to record conditions for storage of durable and of short duration products.

EXAMPLE: SCHEMA OF PROCEDURES FOR REFRIGERATORS*:*

Refrigerators	T°	Contents	Control and records	Corrective activities
Kitchen refrigerator	Λ+ 4°C	Goods for processing in one day or for immediate consumption. This refrigerator can contain defrosted meat kept in closed vessels, cheese, smoked products, yogur ts and milk, and alike.	Daily records of temperature in daily processing table. Control of temperature.	At the raise of temperature by > 3°C call attention of cook, return it to original state. If it is impossible or disadvantageous and repeats more that 3 times a month, it is necessary to ask for special maintenance. Control of inside meat temperature. At the t° > 7°C max Λ 10°C use meat within a day, at the higher temperature it is necessary to remove it, in case of milk products removal is at the t° > 14°C.
Freezers	Λ-18°C	For meat, fish and vegetables. Frosted and/or frozen goods are kept in original package.	Daily records of temperature in daily processing table. Comparison of external thermometer w ith internal.	At the raise of temperature by 10°C call attention of cook, return it to original state. If it is impossible or disadvantageous and repeats more that 3 times a month, it is necessary to ask for special maintenance. At the temperature higher than 0°C immediate use, at the higher temperatures or defrosting of goods the product should be removed. All defrosted or defrosting goods which were not controlled it is necessary to exclude independently on its temperature.
Not washed vegetables	Λ+8°C	Vegetables and fruits kept in original supplies.	Daily records of temperature in daily processing table. Control of outside temperature and its comparison with inside. ell	At the raise of temperature by > 3°C call attention of cook, return it to original state within 2 hours. If it is impossible or disadvantageous and repeats more that 3 times a month, it is necessary to ask for special maintenance. If there are no visible changes on goods, exclusion is not necessary.

Meat and vegetable broths	Λ+2°C	For meat or vegetable broths or ragout.	Daily records of temperature in daily processing table. Control of outside temperature and its comparison with inside.	A t the raise of temperature by > 3°C call attention of cook, return it to original state within 2 hours. If it is impossible or disadvantageous and repeats more that 3 times a month, it is necessary to ask for special maintenance. Control inside temperature of products, at the temperatures of 7°÷10°C cook within a day, at the temperatures >10°C product has to be removed.

2.3.20 Qualification of suppliers

Qualification on the basis of which the supplier is being chosen usually resides in verification of guarantees that is offered by his control system.

Enterprise has to offer sensory, chemical, physical and microbiological characteristics of raw materials (purchase contracts that have to become not only a commercial but also medical tool) and supplier has to follow it. Every supplier is in advanced informed about valuating criteria and corrective steps.

Results of verifications made by responsible person are recorded in tables. These records have to contain used corrective steps as well.

Verification can contain for example control of documents, inspection, measurements and possible analysis.

2.4 PRODUCTION

2.4.1 Preliminary stages

It is necessary to describe processes that will be put to the equal groups or production lines. List of products has already been described in enterprising data. Menu will be added to the sector of nutrition. All products that have the same factors of risk controls can be placed into groups. Every production of operating line is being studied independently beginning with the most important one.

2.4.2 Definition and description of a product or production line

It is required to limit studies with specific types of products/processes.

For company it will be suitable to choose the most meaningful product or type of products, based on produced quantity and complexity of producing cycle, with the goal of creating an example of application of automatic control in the way that common parts could be used also for other producing lines.

Description should contain following parts:

1 title of the product;
2 structure: raw materials, ingredients, additions and etc.;
3 chemical and physical structure and characteristics (solids, liquids, gelatine, a_w, pH, T°…..);
4 adjustments that were made on a product;
5 preparation for packaging and packaging;
6 way of storage and distribution;
7 expected use (other processing, sale to final consumer);
8 destination of product (local market and etc.);
9 period of consumption (studied and showed according to following parameters);
10 labels and explanation for use;
11 microbiological and chemical criteria.

For foodstuff prepared according to new recopies and, in general, in all cases that do not have science data about foodstuff safety, the initial studying phase should expect analytic researches intended on identification of hygiene-sanitary parameters of product.

2.4.3 Identification of consumers

To identify groups of consumers of certain products and mark if sensitive groups are also mentioned.
Extraordinary attention should be aimed at individuals with weaker immune system, children, older people, pregnant women, allergic people and so on (sensitive groups) and evaluate possible unsuitability of consumption and mark it on the label.

The level of risk of a certain product changes considering the type of consumers. This valuation has a great influence on the phase of risk analysis (first rule of Codex).
If the products are not intended for final consumer, it is necessary to mark direct destination.

2.4.4 Description of manufacturing process

1 It is necessary to describe manufacturing process, beginning with receiving raw materials and ending with sending or consumption of final product, enclosing plan of equipment with marked used rooms and cooling boxes.

Attention in description should be aimed at:

2 period and temperature of processing, enclosing possible delays that do not bring process of different processing;

3 quantity and type of processed raw materials;
4 rooms for processing, surfaces and various length of processing

It is crucial to have detailed map of manufactory that contains lay out of equipment and instruments, with clear explanation of use of rooms and cooling boxes.

It is useful to form manual with photographs that illustrates various tools: either the ones that are being used or the tools that are being taken to pieces because of cleaning and maintenance.

2.4.5 Diagram of process

It is crucial to elaborate graphical illustration of processing with a scheme by the help of blocks. Every block can contain more operations that hold the same risks.

Each block contains accurate working regulations, that have already been described, which has to be offered to operating staff.

Flow diagram has to be verified by HACCP group in company to compare theoretical operating with suitable operations in operating company.

Flow diagram is verified with the help of:

1 discussions with workers of manufacturing line;
2 observation of real and good application of working regulations offered to staff, during processing

2.4.6 Restaurants

In the restaurant sector view on HACCP method is different and especially assumes description of complexity of structure, with the help of combination definitions and, consequentially, inside the combinations flows or processing lines will be described.

CHAIN

Way of food preparation and movement (everything inside or outside), examples: central kitchens, terminals, storage rooms and etc.

Thermal chain

Food is being prepared in cooking centers and being served or transferred to other building and there served.

Cooling chain

Raw materials or semifinished goods are being sent out to peripheral cooking centers, preparation usually last 24 hours, never more than 48 hours from the moment of sending out.

Freezing chain

There are prepared frosted foods for sending out. In that case it is necessary to have very complicated

structures with suitable controls of process (time, temperatures and ways).
This type of chain is not recommended for collective nutrition because of nutritional and visual lost of food quality.

PRODUCTION LINES OR FLOWS

Lines of raw material products type *cold - cold*

Before serving raw materials are not subject or are subject to minimal arrangements.

Lines for thermal arrangements

Foods undergo improving arrangements and can go through thermal preparations or freezing of type:

cold-warm

cold - warm - cold

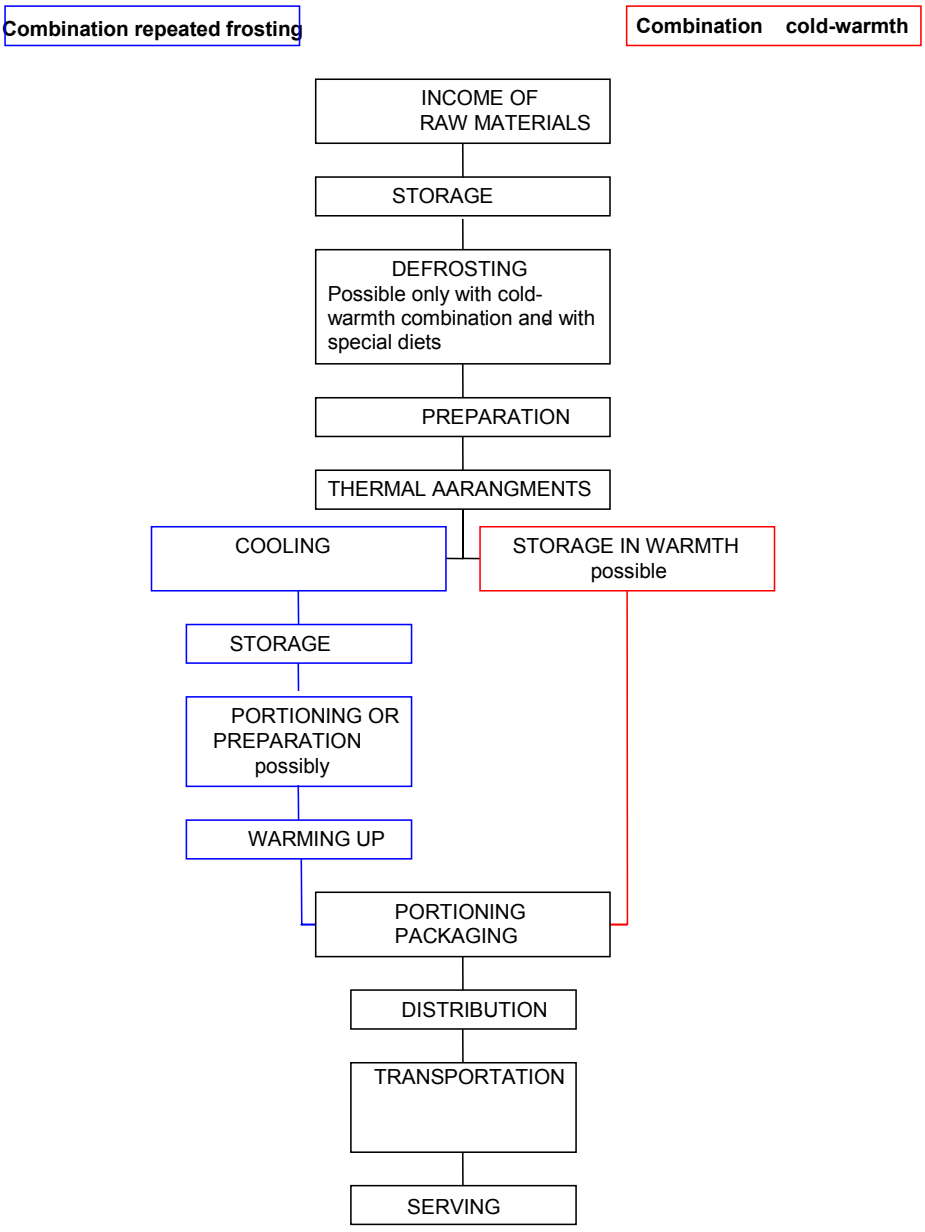
cold - warm - cold - warm

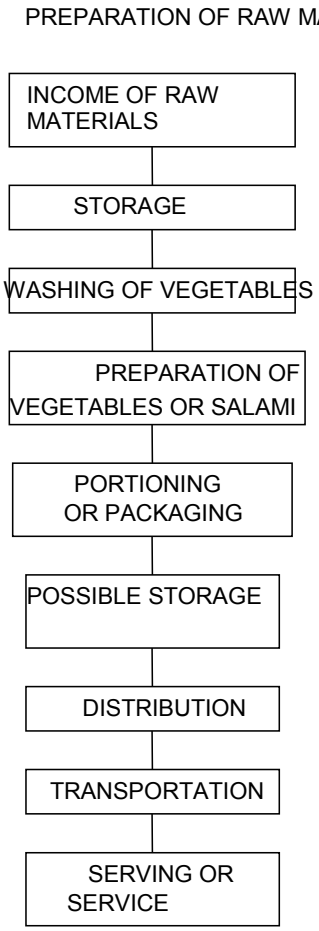
2.4.7 Description of manufacturing process

Observing manufacturing types, it seems that it is very difficult and useless to describe separate preparations.

Useful are way, periods and rooms of operative stages, practically, procedure inside the kitchen during preparation, that works according to organized measures.

With this goal has been defined working program that is being started with receiving from suppliers and to serving or sending out of prepared foods, avoiding crossing with manufacturing lines.


Combination repeated frosting
Combination cold-warmth
INCOME OF RAW MATERIALS
STORAGE
DEFROSTING
Possible only with cold-warmth combination and with special diets
PREPARATION
THERMAL AARANGMENTS
COOLING
STORAGE IN WARMTH possible
STORAGE
PORTIONING OR PREPARATION possibly
WARMING UP
PORTIONING PACKAGING
DISTRIBUTION
TRANSPORTATION
SERVING



2.4.8 HACCP method

HACCP method is divided into seven rules, usually known as Rules Codex Alimentarius:

2. lead *analyses of dangers*;
3. identify *critical control point* (CCP) ;
4. determine *critical limits*;
5. define processes of *monitoring*;
6. define c*orrective activities*;
7. define *verifications*;
8. define *documentation*.

2.4.9 RISK ANALYSIS

The group analyses which risks could occur in each stage of manufacturing process, and on the basis of

flow diagram determines preventive measure that has to be taken for risk control/management.

All recognized decisions (to take or not take risk into consideration) are from the point of view of analysis are realized on the basis of science literature and experience of operating corporation, keeping all steps of applied documentation.

Following are factors, which are necessary to pay attention to for management of risk analysis:

1) epidemiological indicators;
2) microbiological indicators;
3) qualitative requirements for suppliers;
4) ingredients;
5) inside factors (chemical and physical characteristics);
6) procedure of the process;
7) microbiological characteristics of foodstuff;
8) planimetry of rooms, crossings, equipment;
9) packaging;
10) health, hygiene, education of employees;
11) type of consumers;
12) sanitation;
13) notices about use for consumers.

These factors influence foodstuff risk by determining level of risk for consumer.
It consists of series of questions, which have to be applied in each stage of flow diagram.

Difficulties with risk management have basis in determination the ones that can occur, taking into consideration those that have already happened once. This does not mean that at the moment of realizing risk, it has to be taken as CCP. It is necessary to ground the decision for which that certain risk was not taken into consideration. In every case control activities have to be taken in, because constantly there is probability that this risk can happen again without special procedures.

The concept of danger means any biological, chemical or physical factor able to evoke unfavorable medical effect. Under risk we understand valuation of probability of danger occurrence. Severity implies medical dimension of the phenomenon. Preventive and control measures are activities, operations, physical, chemical factors and so on. Operations that has to be applied on every danger with the goal to perform control and management, can be **usual – GMP** (SVP) (aimed at all possible dangers) or **special – SOP** (aimed at management control of special dangers).
Dangers that are to be paid attention to are those whose decrease is important for production of medically non-harmful foodstuff. Dangers that present minimal risk or severity are not taken into consideration.

Biological dangers

Biological dangers come from living organisms in raw materials and change on the basis of quantity and quality during its productive cycle and duration of product consumption. Usually caused by:

- sporulating bacteria;
- not sporulating bacteria;
- bacterial toxins;
- viruses;
- micromycets;
- vermin.

Obtaining good hygienic practice is basis to reduce undesirable microbial contamination, although not always can guarantee absence of pathogenic microorganisms.

Knowledge of characteristics of growth and elimination of main pathogenic microorganisms, found out on the basis of available epidemiological data, will help out with assessment of danger and risk severity and determine activities of control and prevention during processing.

Chemical dangers

Chemical danger is every undesirable matter present in products, as for instance residua from production, preparation, adjustments, from preparation for packaging, from packaging, transportation or from storage of those products or consequently after contamination from the surrounding air.

It can be caused by natural structure of foodstuff, process or contaminant matter during period of product duration and it contains:

- pharmaceutical matter (ex. veterinary medicine, pesticides and phytopharmac);
- matter of anabolic activity or contaminants from surrounding air (hormones, heavy metal, fertilizers);
- contaminants of process (disinfection material, cleaning suppliers, overdosed cleaning ingredients);
- undesirable matter produced during technological process (benzopyrans, metabolites, nitrosamines, chemical changes of fats used during fritting, types of fats (animal or vegetable) and use of antioxidants);
- allergen of foodstuff origin

Dangers of chemical type will be considered in cases where there can be determined and described specific risks. In most cases plans of monitoring and controls of residua made by authorized offices are being observed.

Physical dangers

Attention is put on presence of foreign or radioisotope bodies in foodstuff.

Foreign bodies (wood, glass, metal, bones, rubber/plastic and etc.) can come from raw materials that are in bad operating condition, unsuitable packing material or poor attention of workers. They can be minimized by preventive actions and effective control programs of raw materials, environment and staff.

Bodies of small and very small size are especially dangerous.

Dangers of physical character are taken into consideration in case when its manufacturing cycle helps out with probable occurrence.

Activities to be performed for risk analysis management are:

1) evaluate and identify possible dangers for every stage of flow diagram;

2) evaluate importance with the help of risk and severity assessment;

3) determine preventive activities for control/management

1st activity of analysis: evaluate and identify possible dangers for every stage of flow diagram;

It is necessary to identify biological, chemical and physical potentially present dangers and determine its nature considering the following:

1 **contamination or secondary contamination** (microbiological, virus, presence of vermin, microbial, chemical, physical);
2 **reproduction or outliving** of pathogenic microorganisms or **occurrence** of undesirable chemical matter;
3 **production** of toxins or other undesirable matter coming from metabolism of microorganisms.

Particular biological, chemical and physical factors, that are probably present, are recorded specifically. Some dangers can be grouped only if a source of contamination is similar, for instance eductor contamination, thermal unstable and thermal lasting…)

An example:

A: *Salmonella* spp*., Clostridium botulinum, Listeria monocytogenes*……
B: fats/oil
C: metal, plastic

2nd activity of analysis: evaluate its importance with the help of risk and severity;

If occurrence of danger is considered to be important, that danger must be prevented or controlled that

healthy foodstuff are to be produced.

Importance is valued by:

5 evaluation of **risk** understood as probability of danger occurrence, and in relation to manufacturing process and staff

6 **severity** understood as basis of medical damage performed on consumer and in relation to the agent who determines danger

Evaluation is based on combinations that refer to experience of corporation, to epidemiological data and science literature. Little important risks will not be taken into consideration.

Qualitative model:

Risk predetermines the probability of danger occurrence and is classified to high (H), medium (M), low (L) and minimal or nonexistent (m).

In literature exist models that can be used as tools of common character and that help out in evaluation of danger significance according to quantitative type models, which can work either with the help of definition of danger characteristics or risk categories, taking production type into consideration or looking at probability of foodstuff contamination and severity of illness with utilization of epidemiological data.

1st example: risk assessment on the basis of type of product

According to the NATIONAL ADVISORY COMMITTEE ON MICROBIOLOGICAL CRITERIA FOR FOOD foodstuff, considering presence of danger, are classified to 6 groups marked by letters from A to F.

Risk characteristics:

group A: special group sterilized foodstuff intended for sensitive consumers

group B: product contains sensitive microbiological, chemical and physical ingredients

group C process does not have controlled stages that with certainty would eliminate danger

group D: product undergoes recontamination after manufacturing process and before packaging

group E: abuse of some principles is real during distribution or use, where product becomes harmful

group F: suitable final stage of thermal improvement after packaging or home thermal adjustments does not exist

Categories of risk

Determination of danger characteristics helps to decide category (numbers) of risk from the point of view of quantity (number of dangers).

I) Foodstuff for which danger characteristics are considered non-existing.

II) Foodstuff where applies 1 characteristic embraced in points B to F.
III) Foodstuff where applies 2 characteristic embraced in points B to F.
IV) Foodstuff where applies 3 characteristic embraced in points B to F.
V) Foodstuff where applies 4 characteristic embraced in points B to F.
VI) Foodstuff where all danger categories apply: B,C,D,E,F.
VII) Not sterilized products for risk consumers.

Danger of A characteristic is automatically maximal risk VI, but whatever combination between B and F can have risk VI if designed for sensitive consumers.
On the basis of risk category there are marked four risk types: high, medium, low, minimal.

Quantity of risk

Product	Danger characteristics	Category of risk	Type of risk
	A	VI	H
	+++++ BCDEF	V	H
	++++ from B to F	IV	M
	+++ from B to F	III	M
	++ from B to F	II	L
	+ from B to F	I	L
	none	0	m

2nd example: risk assessment on the basis of probability of foodstuff or raw materials or stages contamination

RISK: probability of danger occurrence.
High, in case when level of contamination is higher than 50%.
Medium, in case when level of contamination is between 11% and 49%.
Low, in case when level of contamination is lower than 10%.

SEVERITY: basis of harm in consumer in case when danger expresses is valued as:
High if result is fatal or expression can have serious chronic extra intestinal forms.

Medium if illness transfers to healthy carriers.
Low if illness is temporary and curing.

Severity in relation to etiological agent changes depending on receiver because of what can be considered both as high and low.

3rd activity of analysis: determine preventive activities for control/management

Preventive and control activities are all activities and actions intended for elimination or decrease of determined dangers up to acceptable rates so that it can be controlled/managed. They are presented with respect of process parameters (duration, temperature, pH, a_w, etc.) maintaining effectiveness of equipment, instruments and tools for measuring and recording, respecting hygienic plans of staff, sanitation of surrounding air and equipment, disinfection and etc.

Preventive and control activities to a large extent influence meaning of danger and make easier determination of CCP, decreasing its number and besides that increasing its effectiveness of self control plan.

For every stage it is divided to:

General (GMP)

Activities or actions necessary to avoid or decrease danger for foodstuff safety up to acceptable rates, considering all implemented and applied in the manual original requirements of process, aim to form optimal conditions of operating, keeping high hygienic level and in specific way decreasing risk rate.

Special (SOP)

Operative procedures with regard to studied and described special dangers have to decrease acceptable rates and especially take into account parameters of process (duration, temperature, pH, a_w, preserving matter, ways of packaging). By specific way they are able to reduce level of risk rate.

Suitable definition of SOP processes makes HACCP plan easier, so allowing control/management of some dangers. In this way corporate sources can be aimed at the most dangerous stages.

In some manufacturing business control/management is connected with only one control factor; it is suitable that determined dangers are managed in CCP, not only in SOP.

Every decision that is given to CCP or SOP for danger management is variable and closely connected with corporate reality. There are regulations for individual corporations, but are applied and evaluated differently depending on cases.

2.4.10 CCP identification

Critical control point is one stage in content of which control will help out with management of given

danger, and is important for foodstuff safety.
Those are stages where it is *necessary and possible* to make control in order to avoid, minimize or decrease danger to acceptable level, relating on safety and hygienic integrity of foodstuff, controlling possible deviations of manufacturing process.
In practice if it is not possible to determine or predict when danger occurs in consequence of loss of control of one phase (CCP), it is important to apply **direct control** to demonstrate management in following phases.

Use of decision tree helps out with CCP determination and also to avoid excessive number of CCP, and at the same time guaranteeing product safety.
Tree is being applied for every phase determined in flow diagram, answering all four questions for every determined danger.
For each determined CCP there will follow series of activities, as for instance definition of monitoring systems, critical limits, documentations that proves CCP control, definition of corrective activities and also evaluation process that is necessary to keep long period of time.
For phases that are not marked as CCP there will be reference to respect GMP/SOP, that is defined as all preventive activities necessary for foodstuff production in hygienic acceptable conditions and which present preliminary requirement for effective application of HACCP system.

Some CCP are identified on the basis of experience and are compatible in same manufacturing types, ex:

1 refrigeration
2 cooking
3 technological adjustments and immediate effectiveness (ex. activity of increase acidity)

Together with decision tree exists another source that, besides simplification of CCP determination, motivates its choice as well. It consists of summarized scheme presented in this case by series of questions.
More precisely it is possible to see that risk level is definitely high. On these grounds it is likely to consider possibility of danger occurrence. In spite of application of preliminary requirements, its management in CCP is needed.

DECISION TREE

O.1 DO PREVENTIVE MEASURES EXIST?

YES NO

IS DANGER CONTROL NECESSARY IN THAT STAGE?

YES WILL STAGE BE CHANGED BY THE PROCESS OR PRODUCT

NO NO CCP STOP

O.2 IS STAGE INTENDED FOR ELIMINATION OR REDUCTION TO ACCEPTABLE DANGER RATES?

NO YES

O.3 DOES STAGE BRING CONTAMINATION OR INCREASE OF DANGER ABOVE ACCEPTABLE RATES?

YES NO NO CCP

O.4 WILL FOLLOWING STAGE BE ABLE TO REMOVE DETERMINED DANGER OR DECREASE THE PROBABILITY OF MANIFESTATION IN UNACCEPTABLE RATES?

YES NO CRITICAL CONTROL POINT

NO CCP STOP

SÚMMARY SCHÉMA FOR DANGER ANALÝSIS AND CCP IDENTIFICATION						
1	**2**	**3**	**4**	**5**	**6**	**7**
Stage	**Found danger**	**Reason**	**Measures taken with preliminary conditions for danger management**	**After application of certain measures, does it concern danger that can occur? If yes, give reasons.**	**If answer on question 6 is positive, what measures is it possible to apply for prevention, elimination or destruction of danger to acceptable level?**	**CCP**

2.4.11 Critical limits

After CCP determination, for each one it is necessary to evaluate limits beyond of which CCP is considered to be outside of control.

Critical boarder is rate that separates acceptability from unacceptability and presents parameter in which given stage should stay to be considered under control.

Those are precise determined rates, specific and measurable; do not refer to the interval of acceptability or norms.

Parameters that are mostly used are chemical and physical, for ex. temperature, time, pH, drop of density (a_w), humidity, acidity, concentration of solutions, concentration of salt content, physical measurements ...., but also subjective parameters, as suitable and unsuitable, under condition of stated criteria.

It is very easy to find out from parameters if CCP is under control, and besides they are realizable, economically accessible and able to guarantee product safety.

Besides critical boarder it is good to define more precise “level of observation” that will help out to foresee tendency of control loss.

Critical limits are based on:

1 experience;
2 legitimate rates (if exist);
3 rates from science literature or acquired from studies with similar products;
4 data collected in corporation from products which are subject of the manual.

2.4.12 System of monitoring

It is needed to define program of observation or dimension of given control parameter with intention to guarantee good management of CCP.

Monitoring systems must be able to foresee the loss of control over CCP, and must offer an answer at **effective time**, so that suitable corrective activity can be applied for repeated gaining of control of process.

The activity of monitoring must assume use of instruments and the former should be compatible, economic possibilities of the company, and technical abilities of operators.

Monitoring can be visual as well, as for example: water boils at the temperature of ~ 100°C, therefore is it enough "to see", that water boils.

It is realized by worker or with the help of automatic or manual systems (on – line, off – line), and it is needed to define **when, how and who** in the manual.

All data from monitoring, on - line a off – line, should be summarized in registration tables, and according to possibility, processed on control forms in order to watch progression of process.

On – line recording systems can be the following: automatic recording of temperature/time (interconnection of these measuring equipments is with equipment of visual or sound alarm and improves effectiveness of activity, guaranteeing possibilities of intervention), off – line systems can be: measuring pH, temperatures and etc.

Microbiological analysis cannot be used for monitoring, because response time is quiet long and sometimes exceeds average duration time of product consumption.

Monitoring in small corporations is especially given by measurements of temperature, time and pH.

2.4.13 Corrective activities

If deviation from the critical limits is found, immediately corrective activities or operations are activated needed for realization in order to return product to safe limits and activities; it is to be applied in order to avoid repetition of deviation. It is crucial to intervene if tendency to the loss of control is found.

Definition and *priori* of corrective activities guarantee rational and correct decision, excluding misled and incorrect improvisation caused by pressing situation.

Corrective activities must be described in precise and systematical way and have to be at workers disposal.

Responsible person must record accepted activities with its period of realization in register of *In discord.*

Example of register of *In discord*

#	In discord/deviations	Data	Corrective activities	Responsible	Time of implementation

It is important to always refer to the register *In discord* when deviation is found, even if it is not CCP.
In relation to the rate of deviation of critical limits, accepted corrective activities can bring revision or HACCP system.

Synthesis defines:

1 means and procedures necessary to eliminate discordance;
2 measures that will avoid repeated occurrence of problem and control that products do not reach consumer;
3 responsible person;
4 loading with products produced outside of safe boarders;
5 written recording of accepted measures ;

Recording introduces:

1 description of found discordance
2 responsible person
3 applied ways and means
4 accepted measures in relation to discord products

2.4.14 Verification

It is needed to realize independent revaluation of HACCP plan being long-term applied and that does not have anything in common with monitoring. Regularity and technique according to which the processes will be applied are defined.
Revision of plan is included in the description of verification.
It is verification that includes two aspects for which producer is extraordinary responsible: Verification and Assessment.

Verification

Verification evaluates effectiveness of system or groups of processes, methods, tests and other evaluations, that together with monitoring accept correspondence with HACCP plan either in actual time or

long-term.
It verifies real application of plan and is applied with predetermined regularity, assures that plan progresses according to expectation and affirms that instructions offered to staff are correctly structured.

Verification brings:

3 revision of documents (monitoring, preventive and corrective activities);
4 direct observation of monitoring;
5 application of corrective activities;
6 direct assessment of function control of monitoring equipment.

Verification of CCP must be realized by a person different from the one that regularly accomplishes monitoring.
Results of verification must be recorded with date, hour, responsible person, given CCP and type of verification (documentary, observation or accomplishment of processes, including calibrating of instruments and equipments), in some case accepted corrective activities, and included products, rates of applied measurements.
It is very important to fill verification table immediately after gaining the data.

Revaluation

Revaluation evaluates effectiveness of system during maintenance of hygienic-medical purposes.
It lies in grouping of all technical-science information in order to determine that HACCP plan, which is correctly implemented, keeps stated goals for management/control of significant dangers.
It is divided into initial revaluation, revision and assessment.

Initial revaluation

It ensures that predetermined parameters in HACCP plan guarantee control/management of the process.
In that phase microbiological and chemical analyses can be useful in order to make sure that in practice it is possible to keep defined goals.

Revision

Revision is similar to revaluation, but it follows after determination and application of the plan.
It evaluates general suitability of the plan.
It is necessary to revise the plan or the parts of it, if the process changes or if significant discord/deviation is found, or if the plan turns out to be ineffective.

Assessment

Assessment is a task of control authority that checks if the corporation activated the processes with the

help of audit for security of danger management/control for protection of consumer health. Regularity of these control activities is in relation to the level of corporate risk.

Sampling

Sampling is being applied in raw materials, semi-manufactured product, and final products.

Activities of sampling require determination of:

- applied instruments
- ways of cull (what way sampling is being done)
- type of sampling (sampling of 2 or 3 kinds, determining n; c; m; M; or more samples by withdrawing of more samples and application of one analysis)
- applied criteria for withdrawing samples, which have to be taken (for ex.: erratic, according to time, number tables random etc.)
- analysis on matrix (physical, chemical and microbiological), on analytic methods (if that is not ISO method, it is necessary to enclose to the manual proofs about comparison with official method), on regularity and boarders of acceptance
- program of tests
- documentation proving date of realized withdrawal of sample and all needed information concerning matrix

In preliminary stage, laboratory analyses are valid help for determination of critical points, for choice of suppliers; in operative stage, they are means of verification for assessment of system effectiveness.

Samples must be realized in relation to the most significant stages of the process, considering wide spectrum of physical, chemical and microbiological parameters.

This way it is possible to process special characteristics that describe the product:

- typical microbial settling;
- preservative factors that interfere with the process;
- time and conditions for keeping the same during commerce.

Type of analysis is being evaluated on the basis of product characteristics.

Parameters for microbiological evaluation:

Total presence of bacteria;

Bacteria of milk fermentation;

Leaven and mildews;

Microorganisms as indicators of hygienic conditions: Enterobacteriaceae, coliform thermotolerant, enterokoks, *Brochothrix thermosphacta* etc.

Pathogens: *Salmonella* spp., *Cl. perfringens*, *L. monocytogenes*, *Staphylococcus aureus*, *Escherichia*

coli, *Campylobacter* spp. etc.

Producer will plan regularity and type of analysis, considering the following:

1 parameters given by the legislative;
2 produced quantities;
3 results of previous analyses (unsuccessful results intensify plan of sampling, on the contrary, constant good results can make the plan more flexible, regarding duties given by the law) .

2.4.15 Documentation

All components of the plan first must document the following:

2 details about risk analysis
3 CCP determination
4 identification of critical points
5 monitoring
6 corrective activities
7 verification processes
8 addition responsibility inside the HACCP plan

As a goal is to manifest:

3 everything that was done and written;
4 that corporation works according to HACCP rules;
5 tendency to the loss of control of some process

Level of complexity of records extremely changes depending on complexity of corporation.
It is not only grouping of useless documents and records, but it must highlight that HACCP plan is being applied according to what was stated, especially considering:

1 results of monitoring;
2 accepted corrective activities;
3 results of verification actions.

CONSTANT DOCUMENTATION: manual of self control (verified, actualized, with date, signed) and register of cases in discord.
TEMPORARY DOCUMENTATION: control tables, documents about interventions, tables of monitoring and etc.
All documentation in written or electronic form must be at official controller disposal. All documentation is a subject to verification, which means that correctness is being controlled with what is written in the manual;

approved document is the one that was announced as operational and actualization of document is given by its revision.

For very small corporations, system of temporary recording can be simplified, expending function of register of cases in discord, considering that everything what is not mentioned is compatible with results of process.

It is needed to keep documentation, concerning deviations and register of cases in discord, because it forms inevitable basis of revision process and activity of supervision from the side of official control authorities.

2.4.16 Table of HACCP application

All described information is summarized in the table:

PRODUCT:_____

STAGE	DANGER	G	R	PREVENTÍVE AND CONTROL ACTIVITIES	CCP	CRITICAL LIMITS	MONITORING OF METHÓD	REGULARITY	CORRECTÍVE ACTIVITIES	RECORDS

PRINCIPLES OF QUANTITATIVE ANALYSIS OF FOOD RISK

THIRD CHAPTER

INTRODUCTION

Quantitative Risk Analysis (QRA) was defined as „the systhematic analysis of the health risks, connected with the specific type of the food product, focused on the estimation of the probability of non-desired health impact after using of the product“ /Notermans and Mead, 1996/.

In the past years have contributed to the evolution of the terminology and metodology, focused on the transmission of the risk analysis principles, by the decisive way several international authorities. In this connection there must be underlined activity, developped by International Commission on Microbiological Specifications for Foods (ICMSF) and World Trade Organization (WTO). Except this, the newest metodical material published by ICMSF takes into consideration some of the arrangements, which are connected with the managing of the global trade under supervision of WTO, introducing some of the criteria for risk evaluation.

3.1 ALOP, TLR AND FSO

For this purpose the concept ALOP (Appropriate Level of Sanitary or phytosanitary protection) was introduced, also called ALR (Acceptable Level of Risk), defined as „level of the protection adopted by the member state, which defines sanitary and fitosanitary level for the protection of the human health, health of the animals and plants or internal health system on the own teritory“ (WTO 1995).

ICMSF has translated this purpose by the criteria defined as TLR (Tolerable Level of Risk), understood as “risk tolerated by the society in relation to other important risks of everyday life“ (ICMSF 2002).

TLR is defined with the respect on the impact on the health of the society, technical possibilities of ealisation, social-economical and political consequences. Such a criteria can be expressed as the number of cases per year, caused by certain food risk on 100.000 inhabitants.

It is important to underline, that TLR is connected with social (and local) factors.

Below is the list of the examples TLR asked from ICMSF:
0,5 cases of listeriosis per year and 100.000 inhabitants;
0,02 cases of cholery per year and 100.000 inhabitants;
0,01 cases of botulizmu per year on each 100.000th inhabitant;
18 cases of local salmonelosis per year on each 100.000th inhabitant;

10 cases of hepatitídy type A per year on each 100.000th inhabitant.
The concept of FSO (Food Safety Objective), defined as „maximal presence of the important micro-organisms or concentration of toxines on the field of health service in the moment of the consumption“ (ICMSF 2002) was introduced, so that the producers/distributors/providers of foodstuff could concretise their necessary activities with the purpose of reaching TLR.

FSO could be determined by competent health authorities, it is presumed, that in European Union the strategic role belongs to EFSA (European Food Safety Authority).

Following the list of some exmples of FSO, asked from ICMSF (2002):
Concentration of stafylokokov enterotoxines in cheeses could not exceed 1 μg 100 g –1;
Concentration of alfatoxines in arašidoch (podzemnica olejná) could not exceed 15 μg kg –1;
Loading of Listeria monocytogenes in food prepared for the consumption could not exceed 100 ktj g –1 in the moment of the consumption;
The presence of the salmonelosis should be lower than 100 ktj kg –1 of the powder milk.

The purpose of the Metodic material ICMSF is to express opinion about FSO application. Especially, when there is for the first time defined FSO for determined food, producers and health bodies could confirm, that such an objective is technically reachable by means of the auto-control. If FSO is proved as reachable, then the producer will apply the activities of auto-control, necessary for reaching of the criterions corresponding with FSO. On the other hand if FSO is not technically reachable, there are two possible hypothesis:

1. adjustment of the process or of the product if possible
2. otherwise the production of such a product will be prohibited.

The system of quantitative risk analysis on quantitative level is focused on evaluation of non-desired health impacts after consumption of the food in relation to determined FSO. Such a system includes three elements/stages: 1. Risk assessment 2. Risk management 3. Risk communication. With those three sub-phases there are conformed three professional persons, which must co-operate with each other as well as co-ordinate their activities: Risk assessor, which by means of the scientific method evaluates non-desired impacts, Risk manager, which is managing estimeted risk and finally Risk communicator, which has to classify the methods and the periods for announcing to the interested cathegories.

3.2 Stages of risk evaluation

Evaluation of risk could be defined as „scientific evaluation of non-desired health impacts, already known or potential, coming out the exposition of the person to the risk“ (FDA/USDA/CDC, 2003).

Evaluation of risk is realised in 4 consequent stages:

Identification of risk,
Assessment of exposition,
Determination of dose / response relation,
Characteristic of risk.

3.2.1 Identification of risk

The phase „Identification of risk“ assumes „identification of physical, chemical and biological agent or foodstuff element, which could cause non-desired health impact“ (FDA/USDA/CDC, 2003).

Identification of risk has two starting points: epidemiologic data about population, which is being studied and data, which concerns evaluation of foodstuff risk.

What concerns microbiological risks, if there could be found out expression of risk suspicion, connected with the presence of pathogen micro-organism in foodstuff product, it is necessary to project experimental researches, which could assume using or evolution of micro-organisms relative to combinations of the most important variable values on microbiological level (predictive microbiology). There exist many typologies of experiments, which could be applied on classifying of the danger, but also in following stages of Risk assessment in the function of research objectives. The criterions of experimental attitude as well as know-how on programing of the experiment and on evaluation of results, are obtained in the part of the document, dealing with the requirements for *Risk assessment*.

3.2.2 Assessment of exposition

The second stage of Risk evaluation – phase „Assessment of exposition“ is focused on „characteristic of the source and the level of expositions amount of the person to the danger“ (FDA/USDA/CDC, 2003).

Evaluation of exposition to microbiological risk includes evaluation of the levels of bacterial contamination and related microflora in the foodstuff in the moment of consumption, as well as the quantity of consumed food during the course throught different cathegories of consumers. Information about consumed food could be gained from the statictics about consumptions, published in the geographical locality of the study, while the microbiologic data could be gained from control activities of the competent authorities or could be found out by

means of taking the samples from foodstuff product in the moment of consumption. ICMSF underlines the tendency, which takes analytical data as health objective, because there is need of the guarantee in the moment of consupmtion and not after the end of determination of the production.

What concerns risks, connected with the presence of chemical agents residua, it is necessary to realise quantitative assessment of exposition to the specific agent, which is expressed as *administred daily intake* (ADI). As the point of origin has to be determined *not observed effect level* (NOEL) or *not observed adverse effect level* (NOAEL), which represent the amount of the xenobiotic, which is not giving the space to important biological impacts (physiological modification: changes of the blood cell, growth of the organ weight, considerable losing on weight., etc.), if is supplied in the diet to laboratory animals during long period (the whole biologic life).

Necessary intro-specific changes require, that evaluation of NOEL in the phase of sensitivity on the toxical impacts of the xenobiotics was realised on more sentitive species. From legislative point of view there are in general sufficient two species, from which one do not belong to hlodavcom (krysa and dog); in spite of this during the experimantation if we sort out between two species more sensitive one, it could not surely be in absolute sense the cause of determined toxical impacts for necessary metabolical differences between laboratory species and species of zoo-technical interest or for the creation of the metabolits. Actually adopted procedure for determination of NOEL could bring to the person not always extrapolabile results.

Consequently after more sensitive NOEL was defined, this value could be changed into animal ADI, which represents amount of xenobiotics, which can be taken during the whole animal life without influence or biological impacts.

ADI for the man is defined so, that animal ADI is devided on disconnective factor = 100, which comes from product 10 (which is aware of intro-specific variability) multiplied by 10 (intra-specific variability).
There are being created hypothesis, that the man is 10 more times sensitive than the animal species, on which experimentation was realised and that the sensitivity of the human species could be changed 10 times more. In spite of this those preventive factors were taken into consideration, for some structures (xenobiotic) was proved variability in inter- and intra-specific sensitivity higher than 10.

3.2.3 Determination of dose / response relation

In the third stage of Risk evaluation there is being realised „*Determination of dose / response relation*“, which is defined as „determination of the relation between fullness of the exposition and the fullness and/or frequency of the non-desired health impacts“ (FDA/USDA/CDC, 2003).

This phase evaluates the importancy and duration of non-desired health impacts, which are potentially consequent after swallowing of the food in relation to dose.
Variability of the effects on the host is depending on different factors, especially from:

1. physiological characteristics of the micro-organism;
2. sensitivity of the host;
3. attributes of the food (presence of the barriers) ;
4. correction of bacillus subletal damages.

Determination of dose / response relation is one of the more critical phases of the project analysis of risk from the reason of experimental data load, which are often turned back to information gained from the products responsible for mentioned food diseases, what of course represents only minimal part of the total sum of the cases.

In this phase there are summarised experimental evidences, which come from the studies about the man and about the animal models, which are comparised with epidemiologic data, with the knowledge and with obtained experiences.

What concerns evaluation of impact dose / response in relation to the chemical risk, it is necessary to take into consideration, that:

1. toxicity of one xenobiotic is in the function of the dose;
2. evaluation of toxicity is being realised mostly throught „living“ studies;
3. for inter-specific variability there is needed experimentation on more animal species;
4. extrapolation of toxicologic data, obtained on animals from laboratory and the man, also if it is theme for discussion, creates actually the only one valid system for evaluaiton of toxicologic risk from the rests present in the food.

3.2.4 Characteristic of risk

In the fourth stage of Risk evaluation there is being realised “*Characteristic of risk“,* which consists of „integration of the danger analysis results, characteristic of the danger and evaluation of exposition and finally in gaining of the evaluaiton of non-desired impacts, which could be expressed on certain population“ (FDA/USDA/CDC, 2003).

Characteristic of risk wants on the basis of information obtained in the previous phases gain evaluation of risk, which is considered as marking of the level of the resulting health impact by determined exposition (e.g. number of cases/100.000 inhabitants per year).

In case of microbiological danger the characteristic of risk could assume comparison between expected risk and epidemiologic data, considering the variability of the impact, which is related to biodiversity of the lines, as well as different division of risk, different levels of contamination and factors, which influence evolution and variability in the response or, if we want, in the response of the host.
Evaluation of risk is so comparised with TLR and in the case, that TLR would not be exceeded, it is necessary to define the activities, which are focused on restoring of auto-control.

3.3 Risk management

„Risk management" is defined as „management of impacts, signes or potentials, which are derived from exposition to risk" (FDA/USDA/CDC, 2003).
This activity can be managed by the same ways as HACCP system, in relation to activities expected from seven principles of Codex Alimentarius, but with close interaction between *Risk communicator and Risk manager*. Such a professional illustrations, which respect own role and specific capabilities, can dispose with modification in the process, eventually activate new risk assessment on the process or modified product.

In managing activities of risk efficacy of control activities can be evaluated by terms „criterion of performance", defined as „result of one or more control measures, applied in one or more CCP for the purpose of foostuff safety approving" (ICMSF, 2002).

In relation to microbiologic risk, performance criterion is in general defined as number of logaritmical cycles, recalling the concept *Decimal Reduction Time* (D) of thermobactery (e.g. reduction 6D of Salmonelosis during heat treatment).

Performance criterions are results of necessary control measures for reaching of specific FSO.

Some of performance criterions were proposed by scientific literature:

1. Reduction 12D *Cl. Botulinum* in non-acid cans (minimum botulinum cook) (Brown, 1997);
2. Reduction 6D L. monocytogenes in prepared frozen meals (Lund et al., 1989);
3. Reduction 6D phylums *Cl. botulinum* in REPFEDs (refrigerated prepared foods with extended durability) (Gould, 1999);
4. Reduction 5D *E. coli* verocitotoxikum in fermented products on basis of the meat (Nickelson and ot., 1996).

Sorting of performance criterions represents especially important tool in activities of risk management. For this purpose there must be studied initial level of risk, as well as modifications induced by the processes (reduction or growth of risk).

Performance criterion, evaluated as sign of amount of subtracted or added impacts to initial risk, must be lower than FSO or at maximum the same as FSO.

H_0 - ∑R + ∑I ≤ FSO; where:

H_0 = initial level of risk;
∑R = sign for sum of risk reduction impacts;
∑I = sign for sum of risk growth impacts;
FSO = objective of foodstuff safety.

Two following examples are dealing with ways of performance criterion application by means of number of one of the coeficients from above mentioned formula.

The first one is related to performance process of beafstakes boiling in relation to risk, which represents surviving of verocytotoxinogene *Escherichia coli*.

Data published in the literature testify, that dose infected by micro-organism can be lower than 100 Ktj g^{-1} (AGA, 1994). Let´s assume hypothesis, that epidemiologic data, related to certain geographical location define, that number of cases/100.000 inhabitants would grow, if filling of the foodstuff exceeded infected dose minimum 1 ktj g^{-1} and let´s assume consequently, that FSO objective would be defined to the level 100-times lower: 1 ktj 100 g^{-1} (for consumption).

If, for example, during activities of risk management, in the RASFF system in frozen meats determined for production of beefstakes, there will be found out presence of verocytotoxicogene *E. coli,* it is also 10^3 ktj g^{-1}, but we can avoid growth (∑I), (because the product is frozen), performance criterion for boiling wil be expressed as following:

H_0 - ∑R + ∑I ≤ FSO; 3 - ∑R + 0 ≤ -2

PERFORMANCE CRITERION: ∑R ≤ 5 (or: 5D).

It means, that for the purpose of FSO objective taking into consideration guaranteeing, determined in appropriate geographic location, management of hardly defined risk must assume at lest one heat treatment with efficacy 5D.

In function temperature of boiling T will be possible to take from the scientific literature the value D_T of monitored micro-organism (expressed in minutes), controlled temperature and to count minimal heat treatment, which must be applied, so that risk would be managed (time/temperature).

The second case is related to performance process of ham maturation, expecially in relation to acceptable level of *Listeria monocytogenes* dosing in the primary substrate.

Data published in the scientific literature testify, that infected dose for this micro-organism is higher than 100 ktj g^{-1} (Buchanan, 1997). Let´s make hypothesis, that epidemiologic data are related to certain geographical location, define, that number of cases/100.000 inhabitants will grow, when loading in foodstuff reaches minimal dose 100 ktj g^{-1} and let´s assume consequently, that FSO objective is related to the level 10-times lower: 10 ktj g^{-1} (for consumption).

If during risk management activities, for example in the system RASFF, we want to evaluate maximal acceptable loading of *L. monocytogenes* in meat legs, it is necessary to evaluate another two coeficients of performance criterion formula, ∑R and ∑I. Let´s make hypothesis, that published data indicate, that in testing conditions of the process there does not exist no treatment of lystery (∑R = 0) and that the level of initial risk can undergo during the process of distribution the same growth as for logaritmical cycle (∑I = 1), maximal loading of *L. monocytogenes* in meat legs (H_0) wil be:

$H_0 - \sum R + \sum I \leq FSO; \quad H_0 - 0 + 1 \leq 2;$

PERFORMANCE CRITERION: $H_0 \leq 1$.

It means that for the purpose of FSO objective guaranteeing, which is focused on appropriate geographical location, management of hardly expressed risk must anticipate maximal loading of *L. monocytogenes* in beef legs on the same level as 10 ktj g^{-1}.

PROPOSALS FOR THE APPLICATION OF THE QAUNTITATIVE MICROBIOLOGICAL RISK ANALYSIS

FOURTH CHAPTER

This part of the document describes proposals for the application of quantitative analysis nutrition risk methodology in relation to manufacturing and distributing system in the Slovak Republic.

The document is based on the analysis of the real case referring to catering in the first part and the second part includes the analysis of experimental methods owing to gathering and processing of necessary data for the nutrition risk analysis. In the first part the attention is focused mostly on application effectiveness of the method, including the total assessment system and the system of risk processing. While in the second part there are data which record the cases mentioned in the scientific literature, as well as a know how of the research planning and choice of the most suitable research way.

4.1. Application of the quantitative nutrition risk analysis

The application of the way of quantitative risk analysis to assessment of nutrition risks processing usually requires ample economic sources and the corresponding professional competence and these are not always at disposal in work groups dealing with HACCP systems planning. Besides, the quantitative analysis process requires longer time than qualitative research and may include proposals for the realization of experimental research carried out in the laboratory or more suitably in the place designed for it.

From this point it is obvious that it is necessary to assess the quantitative risk analysis from the complex point of view, characterised by the current influence of diversity on the risk manifestation for instance on the survival or development of undesirable microorganisms .

The next important case on which it is possible to show the necessity of the quantitative approach is processing of the risks within the system RASFF, for instance referring to identification of activity criteria for the correct risk processing.

Besides these general cases, quantitative research is recommended in the following situations:

- if there is a doubt about the choice or effectiveness of the control for instance if the analytic procedure has not been applied yet,
- if there is not a correspondence on the level of control necessary for reaching TLR, for instance in case of comparing countries with different systems of control,
- if there is an unexpected serious risk for which there are no corresponding scientific data,
- when applying new nourishing technologies which are accompanied by alternative and complementary processes besides traditional acts of compensation.

4.2 THE STUDY OF THE REAL CASE: THE APPLICATION OF QUANTITATIVE ANALYSIS OF THE RISK OF CONTAMINATION BY LISTERIA MONOCYTOGENES WHEN DEFROSTING AND ROASTING MEAT IN CATERING.

4.2.1 Description of the case

At school canteen during defrosting the meat, the surface temperature measured was about 6°C.

4.2.2 Phase 1: Danger identification

Hypothesis: Epidemiological data in Slovakia say that the number of cases of listeriosis for 100 000 inhabitants is rising, if the quantity in meal exceeds the minimum of infectious amount 10^3 ktj g^{-1}.

We assume that FSO is stated on 10 times lower level: 100 ktj g^{-1} (when consuming), e.g. 2 Log ktj g^{-1}.

Physiological characteristics of L monocytogenes

- increase from 0,5 to 45°C;
- pH 4,39 and 9,4;
- from $a_w \geq 0,92$;
- found mostly in meat;
- eliminated by temperatures ≥ 70°C.

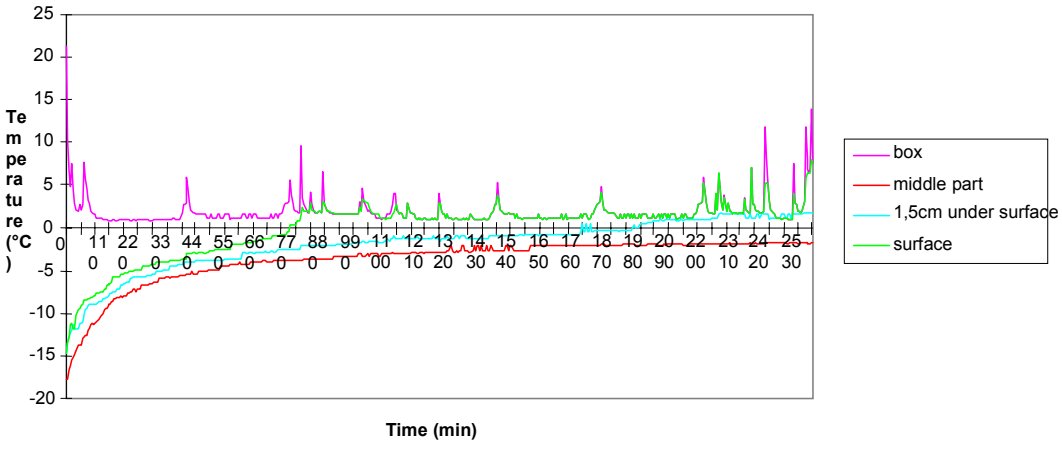
4.2.3 Phase 2: Defining the observation result

At the canteen when defrosting frozen specimen (3 Kg, -18°C) it is assumed that it is stored at the temperatures 2-6°C for the period of five days. By means of data-loggers effective temperatures in the process of defrosting are defined (for instance the surface, inside, medium).

Table 1 gives results of heat measuring during the defrosting process.

Table1: Increase of temperature when defrosting beef meat

TEMPERATURE DEVELOPMENT WHEN DEFROSTING BEEF MEAT



Question: What effect can the exposure to temperatures from 4°C to 6°C for the period of 72 hours have on the development of *L. monocytogenes*?

Results of the surface heat monitoring (pH=5,7):
48 hours at temperature < 4°C
24 hours at temperature ~ 4°C
24 hours at temperature ~ 5°C
24 hours at temperature ~ 6°C

The level of initial contamination danger (H_0)

Hypothesis: Collected data show that there can be 1 ktj g^{-1} of *L. monocytogenes* on the surface of frozen beef meat.

Hypothesis: In case of frozen meat with which freezing is not often done after its processing, it is assumed that the real quantity of *L. monocytogenes* is 10 times bigger than 10 ktj g^{-1} (1 Log ktj g^{-1}), equalling the estimation of the increase of initial contamination danger (∑I *).*

Procedure 1: the research of published data of the phase Lag and the time of creating of (g) *L. monocytogenes* between 4°C and 6°C.

Procedure 2: using the software of assumed microbes development.

Phase Lag and g in the risk heat interval, pH=5,7, for *L. monocytogenes*:
24 hours at the temperature ~ 4°C Lag=103 hours g=18,6 hours
24 hours at the temperature ~ 5°C Lag=86,4 hours g=15,2 hours
24 hours at the temperature ~ 6°C Lag=72,7 hours g=12,5 hours

Conclusion: Published data show that *L. monocytogenes* stays in latent phase during 72 hours at the temperature 4-6°C. Thus: ∑I = 0

Estimation of the reduction of the initial contamination danger *(*∑R ***)***

Immediately after defrosting the amount of meat is carved into thin fillets and is roasted. The monitored process with the data logger shows a rapid increase of temperature and its stabilisation for the period of 1,1 minute at the temperature 64°C.

The way: the research of published data referring to decimal reduction at the temperature 64°C (D_{64}), in beef meat pH 5,7.

In beef fillet: $D_{63,9}$ = 2,2 min (Gaze v 1989).
For 2,2 minutes and 63,9°C, ∑R = 1 (e.g. 1 logarithmic cycle).

Conclusion: Published data show that the presence of *L. monocytogenes* during stabilisation at the temperature 64°C during the period of 1,1 minute is reduced by around 0,5 logarithmic cycles. Thus: ∑R = 0,5.

Computation of the result for FSO=2 Log ktj g^{-1})

H_0 - ∑R + ∑I ≤ FSO; 1 – 0,5 + 0 ≤ 2

The result of observations: 0,5 Log ktj g^{-1}

Conclusion: Monitoring of the defrosting processes and thermal treatment shows that when assuming initial amount 10 ktj g^{-1} on the surface of frozen meat, the result of the contamination risk is much lower with regard to FSO.

4.2.4 Phase 3: Defining the relation dose- response

There are no data available regarding the research referring to people.

Minimum infectious dose MID for *L. monocytogenes*: not known.

It is assumed that at individual dose(Buchanan 1997) the disease of listeriosis starts at MID ≥ 1 x 10^3 ktj g^{-1}.

Assumption: The dose of meat at the canteen weighing 100g.

The amount of *L. monocytogenes* in consumed part corresponds with 100 x 0,5 Log ktj, thus 2 + 0,5 = 2,5 Log ktj (317 ktj).

Consumed dose is thus much lower than assumed MID.

4.2.5 Phase 4: Defining the contamination risk

Number of inhabitants of the Slovak Republic: 5,5 million.

Number of registered cases of listeriosis (2000): 6.

(~ 0,1 prípadov/100.000 inhabitants/year)

No cases of listeriosis from thermal treated meat are stated.

Assumption**:** 0,1% of all cases of listeriosis from thermal treated meat (0,006 case/year in Slovakia).

Assumption: number of doses 100 g thermal treated meat, consumed in Slovakia in one year: 5,5 x 10^7 dose/year (10 dose/inhabitant/year).

Assumption: occurrence of listeriosis mostly in cases of weaker immunity.

Assumption: occurrence of lower immunity in Slovakia = 0,825 million (15%).

If: intake of people with lower immunity = intake of inhabitants,

number of dose/year consumed by the people with lower immunity will be:

$(8{,}25 \times 10^5) \times 10 = 8{,}25 \times 10^6$

Assumption of *L. monocytogenes* implication in thermal treated meat in Slovakia:

Assumption 5% positive

But only 1% positive with amount $> 1 \times 10^3$ ktj g^{-1}

The total number of doses of thermal treated meat fillets with increased content of *L. monocytogenes*, consumed by risk consumers will be:

$8{,}25 \times 10^6 \times 0{,}05 \times 0{,}01 = 4{,}125 \times 10^5 \times 0{,}01 = 4{,}125 \times 10^3$

Thus the probability P that the Slovaks with low immunity may be infected by beef meat listeriosis with increased content of *L. monocytogenes* will be:

P = 0,006 for $4{,}125 \times 10^3$ dose/year, thus:

P = 6 for $4{,}125 \times 10^6$ dose, equals:

$P = 1{,}45 \times 10^{-6}$

4.2.6 Phase 5: Risk processing

Appropriate level of consumer protection (ALOP)

The extent of listeriosis in industrial countries:

2-6 cases/year for 1 million inhabitants (Rocourt and Bille 1997).

The extent in Slovakia :

~ 1 case/year for 1 million inhabitants (2000).

The assumption referring to consequences of thermal treatment of meat (assumption)

0,001 cases/ year for 1 million inhabitants.

The example of ALOP focusing:

50% reduction of consequences of thermal treated meat listeriosis: 0,0005 cases/year for 1 million inhabitants.

Food Safety Objective (FSO)

Recommendation ICMSF (2002):

The content of *L. monocytogenes* in time of intake should not be higher than:

100 ktj g^{-1} (2 Log ktj g^{-1}).

Application of FSO:

Risk Assessment shows that expected results of observations are clearly lower than values of FSO (0,5 Log ktj g^{-1}) and thus have to be considered real and realisable.

4.2.7 Risks analysis and defining CCP (Codex of principles 1-2).

Danger Analysis:

1. Contamination by *L. monocytogenes*;
2. Growth of *L. monocytogenes*;
3. Survival of *L. monocytogenes*.

Flow diagram HACCP:

CCP	Phase	Dangers
CCP	Delivery of frozen pieces of beef meat	1
	↓	
CCP	Storing for temporary freezing	2
	↓	
CCP	Defrosting in the box	2
	↓	
	Carving into fillets	1, 2
	↓	
CCP	Grill roasting	3
	↓	
CCP	Warm-holding	2
	↓	
	Serving	

In the diagram phases in which it is potentially possible to reduce or effectively eliminate the risk are marked with CCP. According to the characteristics of the process the same phase can be given as CCP or GMP/SOP, thus reducing the whole number of CCP.

4.2.8 Defining critical limits and tolerance for each CCP and the monitoring plan development (Codex of principles 3-4).

The plan of monitoring is elaborated which specifies critical limits and tolerance for every check measuring. Using the same methodology as in the phase 2 the data are evaluated H_O, $\sum R$ a $\sum I$ and the whole research is compared with the value of FSO.

The following procedures are prepared:

- Check of input (assessment of suppliers, time limits of delivery, documentation, etc.) ;
- Check of the temperature of meat freezing plant;
- Check of the temperature of defrosting cycle;
- Check of the time/temperature of thermal treatment;
- Check of the temperature of warm-holding.

4.2.9 Corrective activities, verification and documentation (Codex of principles 5-7).

If the value of assumed process result is higher than the value of FSO, it is necessary to apply corrective activities.

They might be defined as:

specific criteria (e.g. minimal thermal treatment: 2 D_{64} for *L. monocytogenes*, e.g.: F_{64} = 4,4 min);

product criterion (e.g. *warm-holding* at the temperature ≥ 62°C)

Finally it is necessary to work out a plan of results verification (e.g. microbiological tests may be included) and the system of documentation which would guarantee gaining the data, products and processes.

4.3. Experimental methods for microbiological risks assessment: study of published cases and methodological proposals.

4.3.1 Assumption

Survival of pathogen microorganisms in the manufacturing processes, contamination after the process, increase in temperature in the distribution phase are topics which increase the interest in methods providing delivery of the product or suspicion of the risk situation before the product's distribution or before it appears on the table of the consumer.

In the case stated above it was necessary to state a hypothesis in relation to risk quantification. Verification of the hypothesis may require a research according to different models in specialised laboratories.

The applied analysis may be organised as a synthetic system in the descending way, taking into account current available methodologies for the analytic systems planning of microbiological contamination risk.

Special attention must be focused on the research *Microbiological Challenge Testing* which represents methods of choice for the assessment of the total process under predetermined conditions.

In the description of this method there are proposals and observations for the choice and preparation of inoculates; these indications may be regarded as suitable for other types of research as well, applicable on quantitative microbiological risks analysis.

4.3.2 Inoculation test

In the phase of planning a new product or on the occasion of assessment and revision of manufacturing process the effect of particular operations the product undergoes may help, e.g. thermal treatment or acidification.

Particularly with regard to microbiological aspect it is often necessary to know the effect of the individual operation on the whole microbiological population of the product.

A help might be the “inoculation test” - inoculating matrixes with the given concentration of testing inoculate of microbe character which is particularly resistant to process influence with the following post-process analysis for verification of the presence of inoculate.
It seems that in the scientific literature there is not a clear difference between inoculation test, microbiological modelling and *Microbiological challenge testing* because these methods assume inoculation and contamination after the process.

We can suggest the following definitions:
Inoculation test – a test aimed at the particular phase control of the process through proving the disappearance of inoculate resistant to this phase.
Microbiological modelling – a study which includes inoculation aimed at creating a mathematical model for the explanation of the microbe phenomenon.
Microbiological challenge testing – a method through which the challenge for survival is sent or challenge for not lessening the function of the microorganism inoculating it and reproducing specific conditions of manufacturing and distributing sphere.

Inoculation test is certainly easier to carry out than modelling or *Microbiological challenge testing* and assumes manipulation with pathogen microorganisms which can be carried out only in specialised laboratories with the means that cannot be used in food processing industry (pilot equipment).

The test application is defined for instance for assessment of particular action in the sphere of system HACCP in cases in which critical limits or critical control points are defined.

By means of the inoculation test it is possible to prove that one phase of the process, applied on the product which has the characteristics of the examined product, is in specific conditions able to eliminate possibly present microbiological contamination risk. This method is used for verifying not active spores in foodstuff which undergo thermal treatment.

Inoculation tests are possible to be used for pathogen survival assessment in specific conditions of the process and survival of the useful microbe culture. For example in yeast products – mainly in dairy and

delicatessen products, assessment of survival and growth of yeast culture may be inevitable – it is mostly found in dairy bacteria, yeast, naturally found in the products or purposely given as starting cultures.

Inoculation test is thus applied where it is necessary to prove the safety of certain operation done in specific conditions. In such a case the emphasis is put not on the effectiveness of the process with regard to specified pathogen, but on microbe inoculate, not necessarily pathogenic, which shows greater resistance to specific treatment.

Finally with regard to conditions specification of the researched process it is not possible to apply achieved results on the assessment of even slightly different conditions of the process.

4.3.3 Predictive models

Predictive models and predictive microbiology in general use detailed knowledge about microorganisms for different microecological conditions with the aim of objective assessment of manufacturing and distributing processes with regard to safety and quality of foodstuff.

The word mathematical model indicates a series of equations where it is defined as a certain prototype, this prototype is not the objective system of the research but it represents it.
The study of modelling is without doubt a difficult research which requires strict keeping of operative protocol carefully examined in order to achieve results divided into following phases: process planning, inoculating, gathering data, fitting of the model and model assessment (148).

In the phase of planning it is necessary to define a proposal for the experiment, specify if the result is represented by the approval of the product safety or its stability. In this phase the process and the product are carefully analysed looking for independent variable, which checks the growth and survival of microorganism better.
For instance independent variables like pH, temperature or a_w, could correspondent with dependant variables like the speed of growth, toxin creation or the time necessary for disappearance of harmful phenomena.
According to the number of dependant variables and the range of the interval representing effectiveness of variability of the variables in the manufacturing process, it is possible to programme the experiment of more variables on different levels: for instance three variables on five levels or five variables on three levels. The level is the number of deviations from the given independent parameter. In the course of applying the scheme defined by Central Composite Design, the central level is defined representing conditions of the common process while oscillations of the variables represent values distant from the centre more or less from zero level (levels +1, -1, +2, -2, etc.).

Prototypes are thus prepared, a series of products specimens designed for research in which there are independent variables purposely arranged in order to represent combinations of variables on different levels according to combination plan of experimental project.
Then prototypes are inoculated by test inoculate, sometimes by the mixture of inoculates and computations are done in the intervals defined by the experimental project.
In the phase of gathering data the analyses results on the prototypes in different intervals are ordered into a scheme.
Thus we can arrange combinations among independent variables (pH, temperature, a_w, etc.) and dependent (scale of growth, toxin creation, the period of harmful phenomena disappearance, etc.)
All the growth curves of test inoculate, created by connecting points representing the functions of the prototype in different intervals (at least 10), there are examined by means of statistic models, for instance model Gompertz (non-linear regression) in order to create equations, explaining growth curves in relation to variables which are differently combined in particular prototypes.

In this phase defined as fitting or adjusting the model, a created mathematical model is confronted with the curves arisen on the basis of combinations of empirical data where all empirical curves, which do not correspond with this model are eliminated.

Equations which underwent the test of fitting are combined with the model which interprets the manner of test inoculate in the course of the experiment through mathematical laws. Polynomial models are often used in which for each characteristic parameter of growth curve (for instance phase lag, acceleration and asymptote) the analysis of regress squared in relation to the growth of dependent variables is applied. After achieving the model it goes to assessment, comparing assumed data with the data measured in examined conditions, on cultivating medium and in the product.

After the model assessment, the model can be used for formulating the assumption of conduct of microorganism in relation to all possible combinations of dependent variables in the interval of the values included in the examined project.

These assumptions can be expressed directly in terms of growth parameters or as a period necessary for achieving the given microbe load or as a number of microorganisms present in the product after certain time interval. This way it is possible to foresee the conduct of the product or microorganism under the conditions of the change of parameters of the process which often characterise the manufacturing cycle. Besides in case of creating a new product it is possible to orientate the creation of the product so that it could to provide maximum protection against pathogenic and harmful microorganisms.

To know the conduct of microorganism at certain temperature and in relation between the growth and temperature enables, when recording the initial number of microorganisms and optimal temperature of growth, to foresee with certain approximation the period of keeping at any temperature.
This assumption is based on different models: Arrheni, Schoolfield and Ratkowsky.
According to the object of study it is possible to define several types of models, the kinetic and probabilistic type (**31**).
Probabilistic models are able to foresee the probability of results at given conditions (in case of *Clostridium botulinum* the result may be the creation of toxin). These models are used with analyses of situations in which there are just two possible solutions for one thing: presence/absence, success/failure, creating/not creating toxin. Besides, the disadvantage is that they do not provide the exact information about the limit when there is the reverse (118). Using these models is mostly useful if it is necessary to assess conditions under which the creation of toxin is possible for instance in case of *Clostridium botulinum*.
Kinetic models enable us to foresee the growth of microorganisms in foodstuff. They are able to offer data regarding the speed of growth of microorganisms, they enable us to state the length of the phase lag or assumption of maximum reachable concentration of microorganisms.

Inevitable conditions for credibility of the model are represented by sufficient number of growth curves on the basis of quality data in order to be able to undergo fitting. Because of this the record of the analysis must be repeated several times and every time the prototypes are prepared which are inoculated and analysed.
Besides, the right choice of interval of the dependent variables is certainly important: the model can be successfully applied in the manufacturing sphere only if this interval corresponds with the effective conditions of changes in the manufacture.
It is evident that information which the models provide are of a predictable character and do not represent certain range of frequency of event, but correspondent with the variability of biological laws.

Finally the model keeps its validity by the matrix and by examined inoculates but mostly the content of the interval of the variables. The change of the process or new creations of the product require a new model creation.
The complexity of this method and the need to gather and analyse a great number of data do not make it easy to spread in food processing industry. In spite of this its application and possibilities which it offers are so significant and interesting that the better future may be foreseen also owing to simplification of the operations by means of informatics (70). The development of special software by American control authorities of United States Department of Agriculture (USDA), (software “Pathogen Modelling Programme”) or the Ministry of economy of Commonwealth (MAFF) (software “Food Micromodel”, **51**) say about the interest of health care institutions in the field of predictive microbiology.

4.3.4 Microbiological challenge testing

Microbiological challenge testing (MCT) is a technique which simulates what happens to foodstuff in the phases of preparation, distribution and manipulation, following the inoculation by the number of microorganisms (32) (3).

The term challenge indicates the action through which the researcher challenges the microorganism to grow or survive in its medium, represented by foodstuff, as it is prepared, treated and distributed in common conditions.

This technique in the last years applied in food processing industry and in research institutes includes inoculation of a microorganism of a given type into food product matrix which is controlled in particular phases of the life of a product.

MCT may follow different objectives: it can be used to define the product safety in relation to pathogenic microorganism development, as well as for defining *shelf-life* in relation to the development of undesirable microbe population or to define criteria of performance of the manufacturing process.

With regard to predictive methods, MCT offers the advantage of direct measuring of the growth and survival of the stem microorganisms in relation to combination of variables of the examined process. On the other hand, this way the assessment of predictive type is not usually possible and thus any change of conditions requires preparation of new tests.

Providing that there is the phase of inoculating, this method requires special measures and equipment designed for this type of research; this is also the reason why before starting the projecting of MCT, it is inevitable to assess carefully the real need regarding the type of responses which we want to get (predictive, direct comparison of one phase of manufacturing or direct comparison of the whole process in predetermined conditions).

Making an experiment assumes the application of the operative protocol divided into sequence phases from projecting the experiment to the interpretation of results (**32**).

MCT is applied when it is necessary to know suitable conditions for limiting or preventing growth and survival of undesirable microorganisms or when there are not well-known mechanisms of lowering the toxins production or when it is necessary to know carefully microbiological picture of the given product in unsuitable thermal conditions.

We can distinguish two types of MCT: MCT of the process and MCT of the product. MCT of the process defines conditions in which the technological process can prevent surviving of undesirable microorganisms.

MCT of the product assesses if the inoculated microorganism is able to breed in the given foodstuff and in that case it defines the level of inadmissible microbe amount for the examined product.

The choice of MCT takes place on the basis of inner characteristics and microbiological profile of the examined product. For this purpose we can take into account the division of foodstuff into two basic types: foodstuff microbiologically stable and unstable.

The stable product is not necessarily sterile but in the common conditions of conservation it does not allow the microbes growth during the period of its business life (non-permissible substratum). In that case it is necessary for the period of the test to be long enough for examination of possible microbes growth.

In case of unstable products the microbes breeding is assumed and thus the test finishes with the development of microorganisms and possible devaluation of the product or the toxin production. In this case it is necessary to study characteristics of the present microbe culture.

In the course of quantitative risk analysis, MCT is applied in order for us to know the dynamics of the growth of pathogenic microorganisms; for this reason one or more product formulations are inoculated by the test stems, which growth and production of toxins is taken into account in different conditions of conservation of the food stuff.

In the process of MCT it is important that the corresponding number of specimens is available to be able to repeat the whole experiment several times. The more times the experiment is done, the more credible the research is.

It is necessary for these experiments to be carried out in laboratories where it is possible to manipulate with the specimens in safe conditions with the presence of experts. The interpretation of results of MCT requires great experience mostly if it contains important results for the public health care system.

Only if the whole procedure of the experiment gives expected results, it is possible to state the assessment of the safety or achieved stability of the product. This assessment will be connected with the conditions of the research and will necessarily have to be carried out outside.

4.5 APPLICATION OF PREDICTIVE MICROBIOLOGY IN MICROORGANISMS GROWTH ASSESSMENT.

Microbiological Challenge Testing (MCT) was applied at quantitative assessment of the influence of the food transformation processes regarding different types of microorganisms.

The following part of the document represents the collection of real cases mentioned in the scientific literature in which MTC was applied for quantitative microbiological risk assessment. These cases are described in particular chapters according to microbes used for inoculation.

4.5.1 *Clostridium botulinum*

Tanaka (**124**) applied MCT to specimens of spreading pasteurised cheese with the objective of assessment of conditions of product safety in relation to survival of the spores of *Clostridium botulinum*.

The author intended to confront two methodologies of inoculation: inoculation “being warm” – it means adding inoculate into warm cheese in the oven, in the active phase and “being cold” – it means adding inoculate in heat shock (80°C during the period of 10 minutes) of the product protected in a closed glass container with the following mixing in an open container.

Five stems of C*lostridium botulinum* type A and B (56A, 62A, 69A, 77A, 90A, 53B, 113B, 213B, 13983B and Lamanna-okra B) were used, inoculated in the form of suspension in distilled water, by the mixture composed of approximately similar quantity of stems. The inoculate added dropwise was equal with the quantity of 1000 spores/for a gram of product.

A complex research project dealing with the assessment of different types of production of spreading cheese with different values of pH a a_w was applied.

The research of botulotoxin in specimens which underwent different techniques of inoculation was done through the experiment using mice by the application of extracts of specimens of spreading cheese.

The author found out an important difference between the results achieved by using different techniques of inoculation, mostly with the production of the products called K a L, with the technique of inoculation being cold, he found out the production of toxin which was not present in the course of technique of inoculation being warm.

For the purpose of finding out the causes of this difference which classified the technique being cold as much more serious than technique being warm, the presence of spores was proved on different parts of the packaging inoculated with the technique being cold.

Tanaka, comparing the results of this experiment with the data of physical-chemical description of different specimens, comes to a conclusion that the result of the method being cold is probably caused by concentration of inoculate on some parts of the packaging, on these spots of the specimens the increased occurrence of the spores with the higher supply of water and smaller concentration of salt in the specimen diluted during the water phase is localised.

While with the experiment being cold a lot of specimens were positive on botulinic toxin, with the experiment being warm only in the group marked Q, in which a citrate was present as an emulsifier, the presence of toxin was proved from the third week after the inoculation. In the group marked M only one specimen out of 60 was positive (after 32 weeks) but later researches did not succeed in explaining such a conduct.

The author suggests the application of the inoculation technique of being warm; in case of positive results with the method being cold, it is recommended to prove it by the inoculation being warm.
Simpson et al. (**2**) applied the methodology MCT on pre-prepared course of meal consisting of spaghetti and ragout – packed, pasteurised and frozen.

The specimens were prepared by mixing 200g cooked spaghetti and 175g packed ragout, gained from distribution network.

At the end in order to keep empirical characteristics comparable with those with the products "minimally processed", the product underwent thermal treatment ("mild technologies", **94**), in order to prevent the survival of vegetative cells, but not to such an extent as to deactivate bacteria spores.

Packed specimens were sunk into water at the temperature 75°C for the period of 36 minutes (the corresponding thermal treatment - 13 D for *Enterococcus faecium*) and then immediately cooled to the temperature 4°C, and finally stored at the temperature 15°C for the period of 42 days.

With the industry products comparable with those which were assessed by these authors, safety and stability were achieved mainly by means of combination of multiple conservative barriers (pH, a_w, temperature of conservation, etc..).

The authors also assessed the risk of toxins production from the compounds of proteolytic stem of *Clostridium botulinum* in dependence on pH, concentration of NaCl and the period of conservation at the temperature 15°C (conditions of slight temperature exceeding).

The inoculate consisting of spores of proteolytic stems of *Clostridium botulinum* type A and B: A6, 62A, 17A, 317121A, CK2-A, MRB, 1B, 13983-IIB, 368B and 426B was used. The spores mixed in the same proportion from each stem, were placed on gelatine-phosphor swabs (bases), inoculated by the specimens and then packed in order to reach the concentration on the level of 10^3 spores/gram of the product (by adding 0,5 ml of inoculate for one specimen weighing 375g).

More specimens with different values of pH and limiting specimens with pH=5,5, were prepared, and also specimens at different concentrations of salt were prepared.

Taking the specimens was done three times, observing botulin toxins on the fourteenth day after storing and then every seven days, biological tests on mice with the extract from the products was done using the procedure comparable with the preceding work.

Besides, empirical characteristics of the product were assessed and analysis of the composition of present gases was done directly under the surface of the packing.

The presence of botulin toxin was found out as well in specimens at pH>5,5 as early as between the fourteenth and twenty-first day of storing at the temperature 15°C, while in specimens with the value of pH = 5,25 its presence was found out only after the thirty-fifth day.
Specimens with pH = 4,4 and pH = 5,0 were shown as negative until the end of the testing period while with the specimens with the value pH = 6,0 toxins were found out on the fourth day.

By the concentration of NaCl with the changed composition of the specimens a considerable effect preventing the production of botulin toxin was shown: with the controlled specimens, with no NaCl added, the presence of toxin was found out only after 21 days, with the specimens where 1% NaCl was added, positive finding was found out only after 28 days, while with the specimens with NaCl > 1,5% added, the presence of toxins was never found out.

Consequently the effect of thermal treatment in the microwave oven for household use was assessed (800 and 400 watts during the period of 5 and 10 minutes); with the specimens containing toxins in which the temperature in the centre was between 85 and 100°C (considerably higher than that at which deactivation of botulotoxic spores is done, e.g. 60-85°C during the period of 20 minutes), constant complete inactivity of created toxins was found out after finishing the processing which lasted 5-10 minutes at half power (400 watts).

The authors finally say that by lowering pH and increasing the concentration of NaCl the creation of important protective barrier against danger which is represented by botulotoxin is created. If we take into account higher concentration of inoculate as it is according to the authors in the limit situation, it is proved that such barriers are normally sufficient in order to provide safety and stability of the product.

Simpson et al. emphasize that in spite of observed change by microwave waves which seems to be sufficient for deactivating the created toxins, it is important to provide safety with these products by means of mentioned barriers (pH, a_w, salt, etc..) and consistent keeping of the correct temperatures at storing.

4.5.2 *Escherichia coli* O157:H7

Escherichia coli O157:H7, as with many other verocytotoxinogenic serotypes (or SLT: *Shiga-Like Toxic*) is responsible for people's serious pathological states as for example states from haemorrhagic colitis to Hemolytic Uremic Syndrome (HUS) or to Thrombotic Thrombocytopenic Purpura (TTP).

Escherichia coli O157:H7 differs from typical stems of *Escherichia coli* according to numerous phenotype characteristics as the absence of growth or reduced growth at the temperature > 42°C, absence of β-glukurnidaze, the inability to ferment sorbite (**139**, **140**). *Escherichia coli* O157:H7 is not particularly heat resistant (D_{145} = 24 s) if it is compared for example with *Salmonella* (D_{145} = 42 s), but survives the temperature -20°C (**139**) and at the temperature 6,6°C it is in the state of slow breeding.

Escherichia coli O157:H7 is more resistant to acids in comparison with other types of *Escherichia* (**126**, 138) and can therefore survive during the period of more days also on acid products as must with pH = 3,8 (**141**) or mayonnaise pH = 3,91 (**20**).

The presence of such microorganisms was found out also at utility animals and thus at foodstuff of animal origin such as beef meat, pork meat, poultry and milk (**50**).

Escherichia coli O157:H7 has the ability to colonise the digestive system at utility animals and at the moment of slaughter it can contaminate muscle substance. This was proved mostly with beef cattle, which was marked as an important source of such microorganisms.

Escherichia coli O157:H7 was for the first time found responsible for the creation of alimentary diseases in 1982 (**50**) and consequently for epidemic regarding the consumption of not efficiently thermal treated hamburgers in fast food restaurants in the USA (some authors speak about "hamburger disease").

The serious manner of pathological phenomena caused by this microorganism causes considerable concern about corresponding control authorities in connection with its possible survival during manufacturing processes and thus transfer of the disease through consumed foodstuff.
In the USA several cases were signalled with considerable clinical importance and several fatal cases were recorded as well. Among the most often infected foodstuff belongs beef meat, mostly in cases where the thermal treatment is not sufficient– as for example in American hamburgers.

In connection with this, control authorities in the USA (United States Department of Agriculture - USDA), using FSIS (Food Safety Inspection Service), organised so called "task force" in cooperation with universities, research centres and representatives of meat and meat products producers ("Blue Ribbon Task Force").

At the end of 1994 twenty-three cases of people recorded as infected by *Escherichia coli* O157:H7 were in the states of California and Washington, following the consumption of salami „San Francisco“ (**127**). In connection with this SDA/FSIS then asked American producers to judge the possible probability of survival of *Escherichia coli* O157:H7 with smoked and fermented products in the USA. As the right method for judging of the manufacturing processes MCT was chosen. It was elaborated in cooperation with experts from the academic field and the field of industry and a protocol was elaborated (work process) about the way of carrying out the tests.(**121**).

The protocol USDA/FSIS requires for the concentration of *Escherichia coli* O157:H7, which will be used in the inoculate mixed with meat, not to be lower than 2 $x10^7$ ktj g^{-1}.

The state of applied inoculate must be proved by taking the specimen immediately after the phase of inoculation using the method of ”recomputation“ to Sorbitol Mac Conckey Agar (SMCA).

At the end of the test it is necessary to do a destruction by 5 logarithmic cycles (5 D) between the computation done immediately after inoculation and the one in complete product; defining the amount of *Escherichia coli* O157:H7 will be done by means of the method of specification on the base and not according to MPN.

Taking the specimens of products in the casing for microbiological analyses is done with two pieces of products taken in he final phase of drying. From each piece of product certain amounts are taken from different parts in order to have an aliquot proportion in the specimen 25g in one piece.

Microbiological analyses are carried out on every aliquot part with the weight 25g, put into 225ml of homogenous solution of the water and peptone. Gradual diluting takes place and then duplicate inoculation on Mannitol Sugar Agar (MSA), earlier incubated at the temperature 42°C. 5-10 accidentally chosen colonies undergo biochemical and serum tests.

In 1995 USDA/FSIS let producers choose from 4 alternatives for the control of possible danger which is represented by *Escherichia coli* O157:H7 with the products in casing or ripened products:

1) Let the product undergo thermal treatment at 145°F (62,7°C) during the period of 4 minutes;
2) Let the product undergo such a treatment which would correspond with destruction at least 5D;
3) To create a programme of so called careful holding (“hold and test”) of completed products observing representative specimens of their production which will be released only after showing negative analyses results.
4) To propose alternative methods which are able to destroy the inoculate of *Escherichia coli* O157:H7 at least on 5D.

These alternatives should be applied both with the products of home production and with those which are imported. Consequently more Italian producers were asked to let their manufacturing processes undergo the verification using the method of MCT.

In fact alternatives 1, 2 a 4 are simply not applicable in case the products are imported from Italy. The application of thermal treatment with already completed product (alternative 1) is certainly not compatible with the required empirical results and it is the same with the most of Italian products of this type imported to the USA.

Alternative 3, e.g. protective blocking of the products directly in the manufacturing hall represents a number of logistic, economic and law measures, which are necessary to be done and during which there is a devaluation of the product and decrease of its weight not speaking about the costs for such analyses and possibly also for law actions.

In the half of the nineties alternative methods were offered to the control authorities in the USA, which corresponded with the destruction corresponding 5D. It was necessary to work out a study for carrying out the analysis and the influence it would have on the producers.

In the meantime requirements referring to 5D were analysed in a very critical way by the associations in the USA. "Blue Ribbon Task Force", in cooperation with the University of Wisconsin (**121**) applied MCT according to the protocol FSIS, in order to asses different processes used by the producers in the USA. It was found out that with conditions depicted in **Table 1** it is not possible to reach 5D, but maximal reduction on 2D can be reached. On the contrary under conditions of the process illustrated in **Table 2** it is always possible to ensure 5D or even higher.

4.5.3 Table 1 The examples of procedures which do not allow to reach devaluation on the level i 5D to Escherichia coli O157:H7

→ Fermentation at 21°C (70°F) with the value of pH=4,6 and drying or storing at 21°C for the period of 7 days, (casings of small diameter);
→ Fermentation at 32°C (90°F) with the value of pH=4,6 and storing at 32°C for the period of 7 days, (casings of large diameter);
→ Fermentation at 32°C with the value of pH=5,3 and storing at 32°C for the period of 7 days, (casings of large diameter);
→ Fermentation at 43°C (110°F) with the value of pH=4,6 and drying (casings of small or

large diameter).

4.5.4 Table 2 The examples of procedures which allow to reach *devaluation on the level i 5D or higher to* Escherichia coli O157:H7

→ Fermentation at 32°C with the value of pH=5,3, thermal treatment and the following drying for the period ≥7 days (casings of large diameter);
→ Fermentation at 32°C with the value of pH=4,6 and storing at 32°C for the period ≥6 days (casings of small diameter);
→ Fermentation at 32°C with the value of pH=4,6 and thermal treatment (casings of small or large diameter);
→ Fermentation at 43°C with the value of pH=4,6 and storing at 43°C for the period ≥4 days (casings of small or large diameter).

USDA/FSIS came to a conclusion that it is not possible to require the devaluation on the level 5D with all these meat products and the fifth alternative of recommendations to producers was added (**121**). It defined the application of the system HACCP which requires analysis of meat mixture before putting it into casings in combination with the process which is able to provide the devaluation on the level 2D, adequate for *Escherichia coli* O157:H7.

With meat products using the technologies used in the United States, before the event with salami „San Francisco“ in 1994, Glass et al. (**137**) focused the attention on the survival of *Escherichia coli* O157:H7 during the process of manufacturing using MCT.

The inoculate consisting of mixture of 5 stems of *Escherichia coli* O157:H7 was used (932 and CL8 coming from clinical cases referring to people; 933 coming from meat; EC 204P taken from pork; EC 505B taken from beef), all of them being present equally.

For the process of inoculation, meat stuff was used (23% beef - 77% pork), given by the producer of meat products. The stuff was inoculated in order to reach the content 4,8 x 10^4 KTJ/g.

After adding the inoculate in the two thirds of the specimens common starter (Lactacel) was used. In the course of the process of drying and ripening the final value was reached pH 4,8 and the proportion of moisture and proteins ≤1,9:1.

In the final phase of ripening, products were vacuum packed and stored at the temperature 4°C for the period of two months.

Escherichia coli O157:H7 survived but did not breed during the phase of fermentation, drying and storing and devaluation on the level 2D was recorded until the end of the examined storing.

Glass et al. at the end of their work state that in the course of the production from fermented meat products containing beef meat it is extremely important to ensure when using initial raw materials that they are not contaminated or just slightly contaminated by *Escherichia coli* O157:H7 from the time when the possible contamination with 10^4 germs could cause its survival during the manufacturing process independently of the use of starting cultures.

Kasrazadeh and Genigeorgis (**17**) elaborated a study of MCT which assessed the growth and control of two stems of *Escherichia coli* O157:H7 with the typical Spanish cheese (Queso Fresco).

Stems 932 and 1083 were used prepared for Brain Heart Infusion Agar (BHIA) and stored at the temperature 2°C before inoculation itself, separately for two stems.

The cheese was prepared in the laboratory following the typical way of preparation and the next day it was inoculated.

Consequently tests for the assessment of physical-chemical characteristics were done with used milk and specimens of cheese.

The inoculation was done with the cheese as well as milk, so there were two separate studies – one for cheese and one for milk.

With the specimens of cheese two stems were inoculated in order to achieve the concentration on the level 10^4 ktj g^{-1}. After inoculating the specimens of cheese they were vacuumed and stored at the temperatures of 8, 10, 12, 16, 20 and 30°C.

With milk the value of bacteria suspension was in order to reach 10^5 ktj/ml and the specimens were stored at the temperatures of 12, 20 and 30°C.

Specimens of cheese were prepared where antimicrobe additives were added in order to research their influence on *Escherichia coli* O157:H7 (potassium sorbite, benzoat sodium and sodium lactate).

The number of *Escherichia coli* O157:H7 with inoculated specimens kept at different temperatures was set in regular time intervals during several months using the soil SMCA.

Achieved results prove that two used stems were not able to breed in specimens of cheese stored at 8°C for the period of two months and for the same period liquidation corresponding the level 2D was found out. And on the contrary a considerable increase with the specimens stored at the temperatures from 10 to 30°C was examined.

Achieved data were also used for elaborating a model study of the microorganisms growth which took into account growth potention of individual stems in examined products following the applied type and concentration of antimicrobe substances.

By means of the complex assessment of the results of MCT and the model study, the authors assume that quick growth of *Escherichia coli* O157:H7 with the specimens stored at the temperatures from 10°C to 30°C is justified, if not enough antimicrobe barriers are created in the assessed type of cheese (the absence of starters, pH nearly neutral, low content of salt and high content of moisture).

By means of model study the effectiveness of antimicrobe substances was compared; sodium lactate showed the lowest antimicrobe effectiveness in comparison with potassium sorbite and benzoat sodium at corresponding temperatures.

Finally the authors proved that typical Spanish soft cheese which underwent tests with the characteristics (pH=6,6, moisture 60% and the absence of starters) is a great substrate for the growth of *Escherichia coli* O157:H7 on condition that the temperature is higher than (>10°C).

The growth of microorganisms may be checked using three ways:

- adding the acid propionate into milk until it reaches the value of pH 5,9 and then adding > 0,3% of potassium sorbite into cheese and storing the completed product at the temperature < 16°C;
- adding > 0,3% benzoat sodium into cheese and store it at < 16°C;
- leaving the completed product at the temperature< 8°C.

4.5.5 *Staphylococcus aureus*

Several cases of intoxication caused by *Staphylococcus aureus* were recorded with dairy and cheese products (**143**). Erkmen (**7**), applying MCT, assessed the possibility of survival of such microorganisms during the production and ripening of cheese Feta.

One stem of *Staphylococcus aureus*, ATCC27690, kept and daily transplanted into Brain Heart Infusion Broth (BHIB), was added into concentration on the level of volume 1, 5 and 10% and starter consisting of one stem of *Streptococcus cremoris* (2% volume/volume).

Modification was done with pasteurised milk at 72°C for the period of 2 minutes before the whey is created so that several inoculates could be used in the extent between 10^5 and 10^7 ktj ml^{-1}.

This way 14 specimens were created: 6 modified by starting culture and *Staphylococcus aureus,* 6 with *Staphylococcus aureus* without starting culture and 2 inspection specimens, and a specimen without a starting culture. Besides, at experimental depiction two different NaCl: 15% and 20% weight/volume were assessed.

The preparation and ripening of cheese was done in the laboratory; in given intervals the count-down of *Staphylococcus aureus* was set, the whole number of aerobe microorganisms and moisture, fats, salts a pH were measured.

These tests were done at the moment of adding inoculate into the whey, at the moment of adding salt, in the phase of ripening and after 15, 45 and 75 days.

Microbiological research observed the growth of *Staphylococcus* using starting cultures or without them; besides, the development of physical and chemical parameters which were able to prevent the growth of *Staphylococcus aureus* was examined.

According to the results of chemical analyses on cheese during the period of ripening the increase of the content of fat was found out (they increased from 15,85% to17,56%), the increase of moisture (from 56,49% to 61,75%) and the increase of the content of salt (from 6,77% to 7,88%).
Percentage content of salt was increasing during the whole period. The values of pH in the phase of placing into forms: pH=4,54 if starting cultures were used and pH=6,33 if they were not used.

The microbiological analyses results proved that with the products without starters the increase was approximately 2,3 D *Staphylococcus aureus* during the first phases of preparation and the decrease during the following phases of ripening of the product.

With the specimens where starting cultures were added the increase was lower (on the level 1,8 D) immediately after the phase of placing into forms; decreasing pH, found out early (in the first 7 hours) and was shown as a great eliminating factor for the growth of staphylococcus. The decrease of pH and increase of concentration of salt even marked the decreased *Staphylococcus aureus* after 22 hours.

Besides, with all specimens (with or without starter) accompanying aerobe microflora bred in he phase of production (increase approximately about 4D with regard to the initial state) and decreased in the phase of product ripening.

From the analysis of the collected data the authors made a conclusion that with the cheese Feta if the product is contaminated by *Staphylococcus aureus* at the beginning, it can enable the survival of microorganism which will be destructed independently of using culture.

The survival of microorganism during the product ripening can be controlled by the parameters of the process, it refers to the value of pH and concentration of salt.

It is inevitable to underline that authors were restricted to quantitative microorganisms assessment but did not assess the possibility of production and resistance of toxin of staphylococcus toxin during the process. Connecting such analysis with the results of MCT it could be possible to achieve information which would have health importance.

Paparella et al. (1999) applied the method of *Microbiological Challenge Testing* when assessing the possible risk of staphylococcus enterotoxin during the ripening of Italian salami.

In this study one stem of *Staphylococcus aureus*, producing enterotoxin A, was inoculated in five periods of manufacture of Italian salami type „cacciatore“. During the ripening and storing of the specimens the production of thermonuclease of enterotoxin, as well as pH and the activity of water was examined.

The plan of experiment was aimed at the assessment of the process conditions of small production company of salamis following the production of meat products in casing when using only pork meat, with quick ripening, with real casings and without adding starting cultures.

After inoculation 1 x 10^3 ktj g^{-1} during 7 days of ripening, conditions for the production neither TNasi, nor enterotoxin were achieved. On the contrary when inoculating 3 x 10^4 a 4 x 10^5 ktj g^{-1} the production of TNasi was started after 24 hours while with the same specimens the production of enterotoxin was found out only after 48 hours. With higher doses of inoculate the production of enterotoxin was found out immediately with thermonuclease.

The data collected at this experiment prove that the tested stem, under the conditions of small production companies of Italian salami products at the initial low contamination corresponding 3 x 10^4 ktj g^{-1} or higher, may reach the value quickly higher than 1 x 10^6 ktj g^{-1} and the production of enterotoxin.

The used stem proved the intensive and early activity of thermonuclease; consequently unlike stated in scientific literature the authors observed that the positive character at examination of thermonuclease does not identify the inevitable exceeding of the level of production of staphylococcus enterotoxin at 1 x 10^6 ktj g^{-1}, but its presence can be found out in salami already at 5 x 10^4 ktj g^{-1}.

Gardini et al. (2002) carried out a quantitative study for the risk assessment of production of biogenic aminos with salamis inoculated by starting culture *Staphylococcus xylosus*, which can be the source of monoaminooxidants.
The presence of biogenic aminos in foodstuff can be considered to be a considerable risk from the point of view of health mostly for the consumers with low monoaminooxidic or diaminooxidic activity, for instance due to taking medicines.

Using the same meat mixture three pieces of salami were prepared by inoculating *Staphylococcus xylosus* S81 from which specimens were taken for microbiological analyses before putting it into casings during drying and during ripening and then after 1, 2, 5, 8, 15 and 21 days.

On prepared specimens with adding histidine and started as a source of monoaminooxidants, relative but clear reduction of the content of histamin was observed; important changes were observed with the content of other aminos as for example putrescin.

These results speak about the content of biogenic aminos in foodstuff either as a result of the whole balance between oxides of aminos or of dekarboxylaktic and aminoacidooxidic activity. For this reason the study depicts the hypothesis about the application of new starting cultures, designed for the reduction of the content of biogenic aminos of fermented foodstuff.

4.5.6 *Salmonella Thyphimurium*

Erkmen and Bozoglu (**11**) applied MCT when assessing the conduct of *Salmonella Typhimurium* of the cheese Feta, it was analysed the same way for the survival of *Staphylococcus aureus.*

The increase and abilities of survival of *Salmonella Typhimurium* were found out with the cheese Feta during the different phases of manufacturing and conservation in relation to the presence of starting cultures with different values of inoculate and different concentrations of salt.

Stem ATCC 11331 *Salmonella Typhimurium* used as a culture having variable values from 2×10^3 to 7×10^4, was added in different concentrations (10, 50 and 100 ml/l) into pasteurised milk, together with the starting culture normally used in the production of cheese Feta (*Lactococcus lactis* subsp. *cremoris* LMAU S69*),* inoculated at the concentration 20 ml/l.

Control specimens of inoculate with tested stem but not starting culture were prepared.
The preparation of the cheese was done in the same way as described in the previous work (**7**).

The amount of *Salmonella* Typhimurium was set and the value of pH was measured in different time intervals: in milk after inoculating, in coagulate after pressing, salting and during the ripening after 15, 45 and 75 days.

Prepared specimens without adding starting cultures showed the development of *Salmonella* Typhimurium 2D as early as during first 7 hours from the preparation; to these specimens the salt was added and it had a limiting effect on the growth of *Salmonella* Typhimurium after 22 hours from inoculation, while pH did not have any influence on microorganisms survival. The considerable elimination of the content of tested stem in the phase of ripening was achieved.

With the specimens of cheese where starting cultures were used, the increase of inoculated stem of *Salmonella* Typhimurium was found out, on the level 1,5D during the first 7 hours after inoculation; the decrease of pH achieved in that time was shown as the next limiting factor for the increase. The combined effect of salt and change of pH caused the decrease of germs of examined specimens after 22 hours from the inoculation; at the end of the period of ripening the next decrease of tested stem was registered.

The complete analysis of the collected data enables us say that the product gained by adding starting cultures had the lowest concentration of *Salmonella* Typhimurium also because the value of pH was lower than 5,7.

The increase of inoculate depends on the combination of the following factors: stating culture and lowering pH, concentration of salt and the time of conservation. The level of inoculate did not have any influence on the survival of *Salmonella Typhimurium*. The authors emphasize the need of using starting cultures in order to ensure the safety of the product.

4.5.7 *Listeria monocytogenes*

Stecchini et al. (**29**) simulated the conditions of contamination after the process, mozzarella was inoculated by *Listeria monocytogenes* (stem ScottA), with the presence of thermostable bacteriocins produced by *Lactococcus lactis* ssp. *lactis* (stem DIP15 and DIP16) isolated in the raw milk.

Mozzarella of home production was inoculated by the testing stem of *Listeria monocytogenes* in order to achieve the initial state 30 ktj/cm^2 on the surface of the product and the concentration approximately 10^3 ktj ml^{-1} in the usual medium. Consequently the product was individually packed into polyethylene sacks containing culture of *Lactococcus lactis* spp. *lactis* – thermal treated and mixed with defatted milk. The specimens were stored at the temperature of +5°C during the period of 21 days and were in regular intervals analysed.

Bacteriocins produced by *Lactococcus lactis* proved bactericidal effect against *Listeria monocytogenes*, causing its false elimination in 24 hours. After seven days *Listeria monocytogenes* continued in its growth but during the period of the experiment they were in the lower levels than with the specimens where starting cultures were used.

Vannini et al. (2002) elaborated the study for risk assessment of *Listeria monocytogenes* at thermal treatment applied on poultry meat with the focus on products covered with breadcrumbs and consequently exposed to cooking - by pre-fritting, and possibility of survival of *Listeria monocytogenes* in covering batter was judged.

The specimens of steaks containing collagen and fat in different percentage were prepared and then they were inoculated by *Listeria monocytogenes,* corresponding the value 6 Log ktj g^{-1}. The specimens were created each from 60g and they were thermal treated corresponding F_{70}^{10} in the time interval from 0 to 4 minutes. Taken specimens were then cooled and the number of *Listeria monocytogenes*, Enterobacteriaceae and mezofyls was defined.

Achieved results prove the protective role of collagen and mutual effect of heat and contained lipids in connection with the curve in the course of heat mortality of microorganisms. The curves of mortality were determined as a result of heating with clearly two-phase course: the first phase variable from 15 to 40 seconds and it was characterised by the loss of viability from 1 to 3,5 logarithmic units while he second phase showed clear logarithmic decrease followed by so called tailing.

Most of all, all the curves referring to the combination of contents 1,5 and 3%c of collagen prove considerable ability to deactivate, more significantly than with the specimens with the same content of fat but with the higher content of collagen (relatively 4,5 and 6%).

The authors stated that the protective effect of collagen against *Listeria monocytogenes* can be caused by the stabilisation effect of cytoplasmatic membrane and at low values of pH. On the contrary the increase of fat could indirectly cause the decrease of a_w, due to higher solubility of fat in the water at high temperatures. Finally the increase of thermoresistance could be supported by rheologic changes caused by the creation of gelatine from proteins at high temperatures.

Paparella et al. (2003) assessed the potential development of *Listeria monocytogenes* gained from smoked salmon of home production from the quantitative point; they used traditional cultivating base (BHI) as well as base which reproduces nutrition demands of the salmon itself (gravy from salmon). NaCl in different concentrations was added to these two media and they were kept at the temperature 4°C, 6°C and 9°C during the period of 30 days.

At the temperature of 4°C the development during the period of storing was not found out while at the temperatures 6°C and 9°C the growth in the salmon gravy was observed, even it was less considerably than base of BHI. At the temperature of 9°C and also with the whole taken inoculated specimens for BHI maximal development was achieved after 4 days while when using the salmon gravy with some specimens this occurrence was observed only after 20 days.

Adding NaCl into two examined substrates there were more noticeable differences in the conduct of particular specimens; mostly the specimen with added 7% NaCl; this effect was proved with all the specimens in salmon gravy but in BHI.

The data, achieved in this study from the beginning of taking the specimens from manufacture equipment and from smoked salmon treated in the common way and from the traditional technology when dry and not the technique of injecting salt solution (wet technique), prove considerable change in conduct of particular specimens in relation to nutrition conditions which are comparable with those which the foodstuff contain.

In another work Paparella et al. (2001) used the method of Microbiological Challenge Testing in order to assess the risk of *Listeria monocytogenes* in the process of marinating anchovies.

Frozen and then defrosted anchovies were boned and washed then contaminated by stems ATCC 7646 and TE010 *Listeria monocytogenes* (the level of inoculate corresponded $1,2 \times 10^7$ and $1,1 \times 10^7$ ktj g^{-1}) they were exposed to the process of marinating and then stored at the temperature +8°C.

After first 24 hours and finishing the phase of marinating, a drastic decline in inoculate with small differences between two used stems was observed. The whole elimination was reached after 72 hours with the stem ATCC 7646 and after 48 hours when using stem TE010. Preliminary results prove that with marinated anchovies the possible presence of *Listeria monocytogenes* in primary raw material does not represent danger for the consumer.

THE CHEMICAL RISK ASSESSMENT

FIFTH CHAPTER

5.1 RISK ASSESSMENT FROM CHEMICAL RESIDUES

The legislation in different European countries relating to toxicological risk connected with consuming residues of the foodstuff has undergone considerable change during last years from different reasons:

1. the necessity to satisfy two contrary needs at the same time: the production of healthy foodstuff on one hand and the impossibility to intensify the methods of breeding in a short time while the use of medicines drastically decreases.
2. the pressure of the public opinion and the consumers movements when preferring foodstuff from the point of view of quality (absence of residues) rather than quantity.

In the last years European legislation including the Italian one did not allow the presence of xenobiotic residues (most of all medicines) in foodstuff of animal origin and required so called „zero residue“. Lately this orientation has been changed because functional development enabled to achieve extremely low levels of sensitivity (ppt or ng/kg) and there is the next improvement.

Besides, different molecules which were rejected for their danger, are naturally present in foodstuff and are created as a result of using physical processes (for instance using heat in the course of smoking or cooking) and their presence is not able to be prevented. As a result at least with some of the molecules the presence of certain tolerable amounts of residues (ADI) whose daily intake is during the whole life without undesirable effects was accepted. On this occasion a series of norms for codification of some of the experimental processes which should emphasize the values of ADI with some xenobiotic substances (medicines, substances polluting the environment, phytopharmatics, etc.) was published.

5.2 INTEREST IN FOODSTUFF CHEMICAL RESIDUE

Since 1960 the interest in residues has referred to the following substances:

Pesticides

Heavy metals

Radioactivity

Stilbenický

Anabolic steroids

β Agonisti

Veterinary medicines

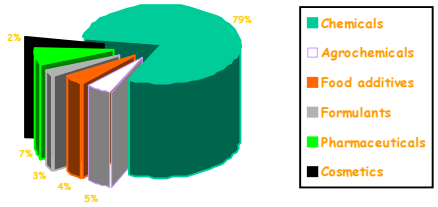
Antibiotics

consequently:

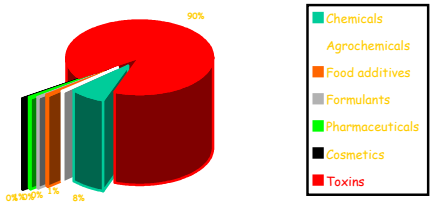
Residues of phytopharmatics
Residues of vet medicines
Hormones
Chemical residues against substances of contamination
Residues of disinfective substances
Residues of cleaning products
Residues of lubricants
Residues of lacquers and other materials for cleaning and disinfection
Mycotoxins
Bacteria toxins
Other natural toxic substances

Foodstuff additives
Colourings
Substances used to modify water
Substances contaminating environment (As, DDT, PCB)
Heavy metals
Dioxins
Substances from packaging and covers
Radioactivity
The state of conservation
Allergens

The complex number and spreading of the type of chemical agents which are dangerous for people.



The complex number and spreading of the type of chemical agents which are dangerous for people + TOXINS



5.3 ITALIAN NORMS

- **Law number 4 from 3/2/61:** forbade in any form and in any way of use the natural and synthetic estrogens as factors of growth and sexual neutralisation of animals whose meat and products from them should be used for consumption by people; this norm enlarged the regulations for imported products as well.

- **D.M. 15/1/69:** enlargement of the above stated prohibition to other categories of substances with hormonal and antihormonal effect (estrogens, androgens, progesterones, tyreostatic substances etc.) and cattle breeders are forbidden to keep and administer them.
- **Law 281/63:** the sphere of feedstuff came under the restrictions with regard to the effect on the health of animals and then of people as consumers of zootechnical productions which catch the attention when using primary raw materials which fulfil the requirements of genuineness and health cleanness as well as prohibition of using estrogens and all the substances with hormone or antihormone effects and specific restrictions imposed at adding antibiotics and other active substances.
- **D.M. 3/11/81**: drastically interferes in the pharmaceutical field, forbidding the sale of medicines which contain stilbenic substances, their derivates, salts and a esters of tyreostatic substances.
- **D.M. 27th August 2004**: sets the maximal limits of residues of active substances contained in the products designed for catering.

5.4 CONTEXT OF EUROPEAN NORMS

Directive 85/469 CE: establishes the prohibition of use of substances with hormonal effect in animal production;

Directive 86/469 CEE: focuses its attention on the research of residues of animals and their excrements, biological liquids also in tissues and organs.

Regulation CEE 2377/90

- Modifies the authorisation in the shop with veterinary remedies
- Defines *maximum residue limit* (MRL)

It approved creation of maximal residue limit MRL, which is accepted as harmful for people's health, e.g. maximal concentration of residues of certain remedy and/or its metabolites which is acceptable in the given animal tissue, milk, eggs, honey and other foodstuff designed for human consumption.

MRL is set on qualitative- quantitative basis of considered residue without toxicological risks for people's health according to the criterion of acceptable daily intake (ADI) or on the basis of temporary ADI which uses additional safety factor. It takes into account also other risks connected with public health and aspects of foodstuff technologies.

It is necessary to emphasize that until these days the norms assume two types of MRL:

- **Definitive MRL:** they refer to molecules with which carried studies and proposed and used analytic methods ensure maximal safety for the consumer;
- **Temporary MRL:** (e.g. temporary, with the given date of expiration): they refer to compounds which require further additional studies.

In cases when as a result of assessment of active pharmacological substance used in veterinary remedies it is not necessary to set maximal residue limit of the health protection of people, the given substance is included into the list which is the subject of the supplement II.

In case that setting the maximal residue limit for active pharmacological substance used in veterinary remedies shows as impossible because of the fact that residues in foodstuff of animal origin represent risk for the consumer's health independently of the limit which was set, this substance is included into the list which is the subject of the supplement IV. *Giving substances which are stated in supplement IV to animals designed for animal production is forbidden in the whole EU.*

Regulation CEE number 508 from 4th March 1999 which changes the instruction CEE number 2377/90, with constant updating sets maximal residue limits (MRL) of veterinary remedies in foodstuff of animal origin (A.O.).
In G.U. of EU there are different supplements in connection with this regulation with the record of MRL of veterinary remedies found in foodstuff (A.O.).
For all remedies which are not recorded in regulation CEE furthermore "zero residue" is valid.
Besides, numerous European regulations were accepted which strictly forbid the use of stilbenic substances (DES and similar), tyreostatics and any active principle with estrogenic, androgenic and gestagenic activity to anabolic purposes. Animals which possibly underwent this type of processing cannot be slaughtered and corresponding products form them must be destructed. In spite of that it is allowed to use compounds of hormone type (with the exception of stilbenic and/or tyreostatic substances) in emergency cases when their use is inevitable (f.e. infertility) and under strict control of veterinary doctor.

Regulation EK 23/96 (D. Lgs 336/99) + Dekr. EK 747/97
The plan of control of national products in the course of residues examination

- Authorised medicines
- Forbidden medicines
- Contaminating substances

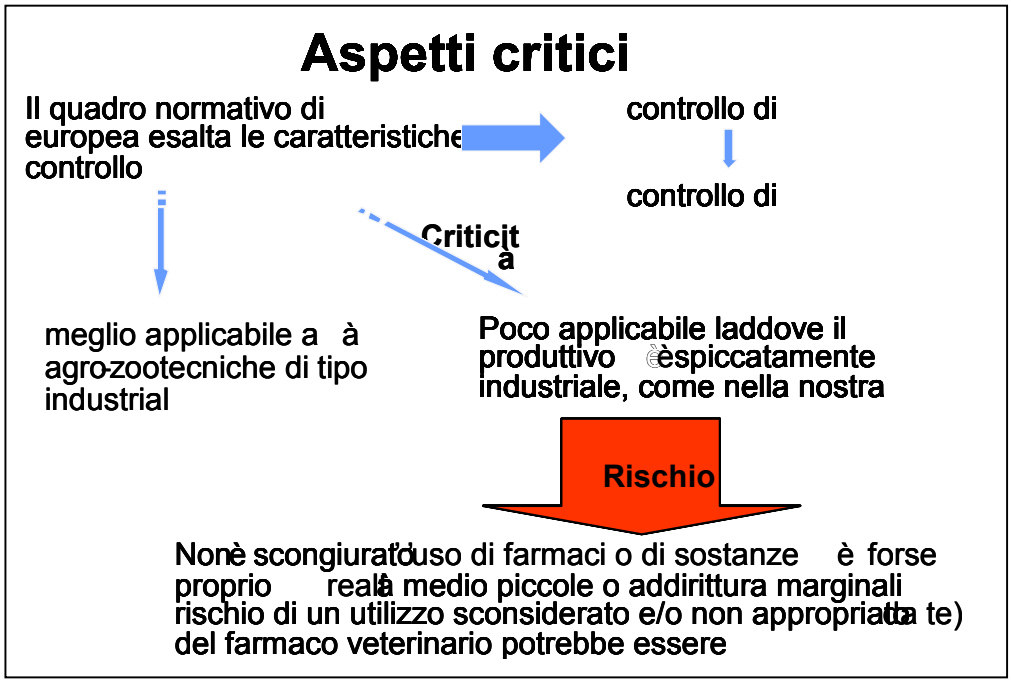
The responsibility for the complete product may refer to different subjects: besides authorities carrying out the control on the first place there are persons responsible for primary production such as breeding and secondary production such as gathering and transformation of the production as well as the company vets themselves. Each phase of this chain must correspond with the guarantees for the product in the course of the following phases.

Regulation number 2375/2001/EK of the Council from 29th November 2001 and Regulation 102/2001/EK: law limits of dioxin were introduced contained in different foodstuff and feedstuff. The producers must carry out analyses in the regime of self-control of the chain in foodstuff production and in raw materials (in cheese products from powder milk of foreign origin or milk produced by animals which were fed by contaminated

feedstuff) and the sale of these remedies, which contain stilbenic substances and its derivates, salts, esters of tyreostatic substances for veterinary use is forbidden.

5.5 NEW MANUFACTURING ORDER

Accepting these norms brings new aspects. It is important to set the whole agricultural, zootechnical and food processing chain as an object of monitoring and not only individual phases which have been so far judged individually as actions not dependent on each other. They were isolated actions which did not belong to one common process. At present the zootechnical production is being looked at as a complex process in which tracing up of the products and their components in all different phases of production, the responsibility of producers and authority controls are elements, which are inevitable for a reliable mechanism which even if it does not ensure the concept of zero risk, reduces the risks for consumers to minimum. It is obvious how in this context approving of the norms follows usage of medicines in breeding practice and in extreme summing the development of the breeding itself. However, this system shows certain restrictions in the course of its real application as it is stated in the following scheme.



In the last decade the legislation referring to residues of the foodstuff has been considerably changed: It is not spoken about „zero remnant“ in foodstuff but about ADI, whose everyday intake during the whole life is without undesirable effects for the consumer.

The concept of „zero remnant“ is invalid due to increased pollution of the environment which is to blame for the presence of contaminating substances in foodstuff as well as due to using medicines in zootechnical breeding and besides prophylactic -therapeutical purpose also for auxinic purpose.

5.6 Residues classification

The residues are divided into three groups:

5.6.1 The residues of accidental contamination

There are different xenobiotic substances - those which can be present in food products by accident or by mistake as a consequence of “contamination” of animals from direct or indirect causes (pollution of the environment, pharmacological influence, contamination of feedstuff, etc.).

These substances are divided into:

- ***Contaminants from the environment*** caused by industrial discharging,

phytopharmatics heavy metals , compounds of organic chlorides, radionuclides, etc.)

- ***Substances with medicated effect*** as medicines, integrators, auxinic substances, etc.;
- ***biocontaminants*** as mycotoxins;

5.6.2 Residues of purposely added substances

They are connected with substances knowingly administered at productions from utility animals. They include:

- **substances used for prophylactic-therapeutical purpose** (antibiotics, chemotherapeutics, medicines against parasites, etc.);
- **substances used for illegal purpose** by the breeders in order to improve the performance of the animals (natural and synthetic hormones, anabolic steroids, cortisones, tyreostatic substances, beta-agonisti, etc.)
- **residues of added substances**: coming form using substances which are knowingly added into food products in order to improve their quality, conservation and health effects known better as “additives“;

5.6.3 Residues of newly created substances

To this category belong the residues which are created in some foodstuff as a consequence of the treatment of the physical character (thermal, radiation, etc..) or chemical character with recognised mutagenic activity. As an example we can show nitroaminos and heterocyclic aminos, which are created by pyrolisis of proteins and some amino acids (foodstuff cooking) and polycyclic aromatic carbohydrates (PCB) which are created by the process of smoking.

The decision to define residues limits acceptable in foodstuff enable their free movement within EÚ and the return to the most traditional manufacturing process is supported and an important reduction of medicine use and additives at the side of producers. In spite of that, even if for some residues the tolerable residues levels were defined at present we do not know possible toxicological effects coming form current intake of residues of different molecules by means of every day diet.

5.7 RISKS AND EFFECTS TYPOLOGY

Intake of residues of xenobiotic substances present in the foodstuff may expose people to ***direct*** and/or ***indirect*** toxicological risks.

5.7.1 Direct toxicological risks

Direct toxicological risks are those which come from intake of residues of chemical substances contained in foodstuff. They can evoke toxic symptoms, which burden different organs, hypothetically connected with intake of xenobiotic substances even though only in a little residue amount (in general ppb and/or rarely ppm). In such cases it is very hard to state the proportion dose/effect between consuming certain undesirable residues by means of some foodstuff and of a toxic character.

Classic **examples** of toxicity are:

- from sulfonamids and aminoglykozids;
- syndrome of depression from tranquillisers;
- syndrome of irritation from aminoglykozids;
- nervous system disorders (parestesia, ataxia and sight defects) of Japanese fishermen from Minamata who ate fish containing a high amount of methylmercury;
- urgent intoxication by consuming meat products (beef liver) containing residues of clenbuterol (0.2-0.5 ppm), beta-agonista, which is illegally used as auxin, its characteristic symptoms such as anxiety, headaches and dizziness were described in Italy, France and Spain;
- allergies usually restricted to skin problems (reddening, itching, oedemas) and accompanied by headaches and dizziness, in more serious cases there can also be enlargement of lymphatic nodes and joints, bronchospasm, but only sporadically there is Quick's oedema and crisis/shock of anaphylactic type which can cause death. Allergic symptoms can be assigned to molecules which are naturally present in foodstuff (eggs, meat, milk, fish, bark-boring beetles, etc.) and only a very little amount may be assigned to intake of medicines residues (antibiotics or chemotherapeutics) or other xenobiotics.

Among xenobiotics which can mostly be found in the genesis of allergic forms are penicillins (f.e. spread through milk as a result of mastitida therapy of black cattle or sows in intramamilar way). Definitely lower are allergic properties of: aminoglykozids, sulphuryl amides, tetracyclines and macrolides.

5.7.2 Mutagenic effects

Mutagenic effects are responsible for damage on genetic cell property caused by different xenobiotic substances mostly:

- *mycotoxins* (A, F, M, ochratoxin A, patulin, toxin T-2, zearalenon, etc.)
- *residues of additives* (formic acid, benzoan acid, ortofenylfenol, sulphuric dioxide)
- *newly created residues:* nitrosoamines, polycyclic aromatic carbohydrate (such as benzopyren) which are produced in the course of smoking and heterocyclic aminos (IQ, MeIQ, MeIQX) with the highest mutagenic ability which are produced in the course of cooking foodstuff.

5.7.3 Teratogenic effects

Teratogenic effects are responsible for the changes on embryonic level. The risks of toxic effects on embryo and foetus during the ”critical period of pregnancy“ because of residues which are present in foodstuff are small and refer mostly to medicines. It seems that the following have mutagenic activity:

- *Residues of antiparasitic* benzimidazols found in milk (kambendazol, parbendazol, oxyfendazol, albendazol, febantel, etc.), responsible for malformation of skeleton of sheep;
- *DES* (taken by women during pregnancy may evoke wrong formation on boy's genitals as hypoplasia of genitals).

It is necessary to emphasize that teratogenicity of one molecule unlike mutagenic and carcinogenic effects is dependant on the dose.

5.7.4 Carcinogenic effects

It was recorded that numerous xenobiotics are able to evoke cancer creation with laboratory animals and people; therefore in spite of disagreeing opinions it would be necessary to prevent the presence of any carcinogenic substances in foodstuff.

Evoking the creation of tumour at the side of xenobiotics may be summed up into two phases:

a) **1st phase** or neoplastic transformation when DNA cells are changed;
c) **2nd phase** or neoplastic development when changed cells breed until the creation of real neoplasia;
d) **3rd phase, advanced,** cancer cells become wrong and proliferation is in the state of invasion of near tissues and metastases (Yassi, Kjellstrom, de Kok, L. Guidotti, Enviromental health, Oxford press 2001).

5.7.5 Distinguishing carcinogenic effects

Carcinogenic xenobiotics are on the basis of their operational mechanism divided into: ***genotoxic*** and ***epigenetic*** carcinogens. Genotoxic carcinogens directly react with cell DNA and often have mutagenic properties. Epigenetic carcinogens do not have any influence on DNA but they are able to evoke the creation of neoplasia by means of: **a) cytotoxic effects,** causing chronic harm of tissues, **b) hormone effects**, **c) creation of especially reactive metabolites**, **d)** promoter act to neoplastic cells existing in quiescent condition. The risk that the consumer may consume together with the diet also residues of carcinogenic effects cannot be underestimated . The attention is focused on the following categories of substances:

- medicines for veterinary use;
- molecules used as "promoters of growth“;
- contaminants from the environment;
- biocontaminants;
- residues coming from added compounds;
- newly created residues.

It is necessary to emphasize that in the last years the risk of carcinogen from medicines for veterinary use was reduced due to the current legislation that assumes performing toxicological research aimed at the evidence of carcinogenic effects with different pharmacological molecules. In spite of that until recently “promoter “ remedies were freely available on the market, containing carcinogenic substances (arsenious oxide) which may be gathered in the tissues of liver and kidneys.

Until this day there is the illegal use of different substances with carcinogenic effect with the cows for the auxinic purpose:

- **DES and stilbenic derivates** (epigenetic carcinogens able to lead mostly in laboratories to the beginning of neoplasia of women’s genitals which can be transferred to children);
- **“growth stimulators"** with hormone effect (natural estrogens, steroids, anabolic steroids and zeranol);
- **contaminating substances from the environment**: organic halogen derivates (DDT, PCB, etc.);
- **biocontaminating substances,** represented mostly by *mycotoxins:* aflatoxin B_1 AFB_1, found mostly in foodstuff of crop origin as peanuts and flax as a nutrition for cattle, a strong carcinogen with hepatic tropism, its hydroxil metabolite M_1, which can be found in milk and okratoxin A with considerable nefrotoxic properties.

5.7.6 Indirect toxicological risks

Indirect toxicological risks are those which are responsible for bacteria resistance (AR), the cause of therapeutical failures when treating different infectious pathological demonstrations of people and animals supported by microorganisms. The antibiotic is aimed at the selection of the forms resistant to the antibiotic at the same time reducing the number of bacteria which do not have AR. For this reason adding sub-therapeutical doses and chemioantibiotics into feedstuff was considered to be responsible for the selection of bacteria stems AR which affect people mostly by means of foodstuff on the basis of meat and when consuming milk and eggs.

5.8 TOXICITY PARAMETRES ASSESSMENT

Dangers connected with the presence of chemical substances residue in foodstuff require quantitative defining of organism exposition to specific agents (ADI). For this purpose an estimation NOAEL is carried out according to the criteria described in the general part and it is transformed into animal-like ADI.

This value defines the maximum daily intake of chemical substance which does not have the effect on health after exposure which lasts during the whole life. ADI is usually NOAEL divided by uncertainty factors (UF).

NOAEL

ADI

Table: uncertainty factor

Factor	Commentary
10 X factor	Applied on data achieved from experimental studies controlled with prolonged exposure, this protects the sensitive part of people.
100 X factor	Applied when experimental data about people do not exist or are not adequate but valid results of studies are available from trying doses on one or more animal species, this takes into account extrapolation species-species.
1000 X factor	Applied when there are no studies on the basis of doses or data about people with urgent exposure and there exist only insufficient results of experiments on animals, this is applied in order to take into account extrapolation species-species at high doses and long-term and short-term effect.

Specific experimental protocols are applied on laboratory animals for the purpose of these parameters:

- chronic toxicity: by means of a study carried out during the period of 90 days orally with two species: one rodent (it is usually a rat) and one a non-rodent (it is usually a dog);
- fertility: by means of a study carried out on two generations of rats. Two litters are judged with every generation: if the toxicity appears at lower dose, the third generation will be included;
- teratogenesis: by means of a study carried out on two animal species;
- mutagenesis: by means of a directed study which requires a series of tests;
- carcinogenesis: by means of a specific study carried out only if the suspicion of carcinogenity of the given substance (structural analogy with known carcinogens, the results of mutagenesis).

The study on toxicological assessment of people takes into account undesirable effects which may manifest as follows:

- short-term (urgent): within one day from the exposition;
- medium-term: from 30 days to 6 months;
- long-term (chronic): from 6 months during the whole life;

in relation to exposition caused by foodstuff (through meals and drinks), reasons connected with dwellings (parks, towns, highways) and working reasons (industrial, agricultural, etc.).

It is necessary to study the metabolism of the substance which we took into account for the purpose of possible toxicity assessment with substances produced by the exposed organism and the organ which is the target sensitive to the presence of xenobiotics.

Possible synergic effects caused by current exposition to different undesirable substances complicate the risk assessment (development of hepatic neoplasia cannot be directly or indirectly connected with the exposure to effects of aflatoxin or ethanol or some virus causal organism). Epidemiological observations must be carried

out to be able to define the proportion dose/effect in relation to eating habits as well as the environment around dwelling and working place in different geographical zones.

Observations of this type are in general led by the groups of experts coordinated by International Agency for Research on Cancer (WHO/IARC).

5.9 RISK MANAGEMENT COMING FROM CHEMICAL RESIDUES

5.9.1 Monitoring criteria

For the purpose of consumer's health protection the need to monitor the level of concentration of undesirable chemical agents arises and we take into account the fact that the concept of "zero residue" is not valid any more because of pollution of the environment which is responsible for the presence of contaminating substances, as well as because of using medicines and phytopharmatics in animal production line. So:

- What are we looking for?,
- Are examined substances able to guarantee the control of potential danger for the consumer?,
- Which other possible compounds could be researched in corresponding category?;
- On what level are we searching?;
- What is the required level of concentration?;
- How are these observations carried out?;
- Is there a possibility of quick screening?;
- What are current possibilities (number of specimens, terms for responses?;
- Is the offer of the laboratories which carry out the analyses in correspondence wit the expectations of the user (terms, certification, etc.)?

Institution organs which deal with arranging plans of authority controls and monitoring define the number, typology of observations which are necessary to be carried out, etc. and the following plans of control are elaborated:

- The plan of residues monitoring;
- The plan for pesticides monitoring;
- The plan of zootechnical products monitoring.

At the moment when the presence of forbidden residues is found out (cat. A Regulation 2377/90/CE), the activation of quick alert system is started if *Minimum Required Performance Level* (MRPL) is exceeded and limits which are all the European laboratories of control able to guarantee analytically.

The quick alert system is activated also when allowed residues limits are exceeded (MRL) referring to substances for which this limit is assumed (cat. B Regulation 2377/90/CE).

5.10 Examples

5.10.1 Contamination by medicines - Chloramphenykol (CAF)

- Discovered in 1947 in cultures of *Streptomyces venezuelae;*
- Wide-spectrum antibiotics;
- Irreversible toxic effects incompatible with the consumed dose;
- In veterinary medicine it is an unauthorised antibiotics;
- It is used illegally in fish breeding, chicken breeding and rabbit breeding.

5.10.2 Chronology of alert:

- Summer 2001 – In one European control laboratory the presence of residues of CAF was found out in fish products coming from China and Thailand: alarm in Europe;
- November 2001 – Reference laboratory of EU (CRL) expresses technical opinion on CAF:
- *Zero tolerance;*
- *Lowering the limit of defining methods used in control laboratories to 0,0003 mg/kg (0,3 ppb);*
- *January 2002 – block of import form China*.

Researching residues of CAF was exceeded to other foodstuff and:

- February 2002 – alarm in Europe (UK) for CAF and streptomicin in Chinese honey;
- March 2002 – alarm in Europe (UK) for CAF in Chinese honey beebread;
- Immediate block of import of these products from China;
- Exceeding analytic parameters defined for fish products with honey (LOQ 0,3 ppb).

Consequent exceeding of analytic parameters to trout which unexpectedly shows residues of CAF even on the very low level. Such occurrence may be connected with:

- Possible wrong production;
- Possible contamination of feedstuff (fish-flour).

The presence of residues of an unauthorised medicine always requires high level of attention.

5.10.3 Contamination caused by the environment

Regulation (CE) number 466/2001 of the Commission from 8th March 2001 defined maximal content of contaminating substances such as lead, cadmium and mercury in foodstuff and assumed or proved other limits for nitrates, aflatoxin and monochlorpropandiol, which can be found in consumables (supplement 1) whose application takes into account the following assumptions.

Plants with broad leaves are the main source of nitrates which can be transformed into nitrites and nitrosoamines (carcinogenic).

Scientific council for the people's nutrition EK, interpellated by the European Commission stated that "the total intake of nitrates is in general under the tolerable daily level" and that "anxiety relating to the presence of nitrates should not discourage from the intake of vegetables because vegetables have a fundamental nutrition function and play an important role when protecting health". It does not reduces the fact that limits must be set which existed before with the regulation number 194/1997 and which were more or less proved.

The council for nutrition stated that aflatoxins develop under the conditions of high temperature and moisture and they are genotoxic and carcinogenic substances.

There are different types and aflatoxin B_1 is the most toxic one: the Regulation set limits strictly which begin with the absence in milk to maximum two organisms for one kilo of peanuts. With other aflatoxins a bit higher limits are assumed. The following norms define the levels of aflatoxin M_1 in milk.

Lead may prevent developing process of recognition and intellectual abilities of children and it also raises the blood pressure and the occurrence of cardiovascular diseases of adults.

The limits set in Regulation refer to milk, baby food, meat, fish, cereals, legumes, vegetables, fruit, oils, fat, fruit juice and wine and span from 0,02 to 1 mg for 1 kg of the product.

Cadmium which gathers in the human body can cause the dysfunction of kidneys, harm the skeleton and cause insufficiency of reproduction system not excluding carcinogenic effects. Limits assumed in the Regulation refer to many products and span from 0,05 to 1 mg for 1 kg of the product.

Mercury can cause disorders of normal development of children's brain and on the higher level it can cause neurological disorders of adults. It can be found mostly in fish products and the regulation sets the limit 0,5 mg for 1 kg of product with the exception of some fish as sea perch, lophius piscatorius, palombo (species of shark), etc., where the limit is 1 mg for 1 kg of the product.

5.10.4 Metals residues in foodstuff

Metals which are found in foodstuff can be divided into three categories:

- **Chemical elements** in general are present (**Fe, Zn, Cu**);
- **Oligoelements** (**Co, F, Mn, Mo, Se**);
- **Heavy metals** and elements of toxicological importance (**Pb, Cd, Cr, Hg, As**);

The presence of them in foodstuff may represent a serious problem for the people's health: serious cases of intoxication by mercury and cadmium were recorded in the past in Japan because of spread consumption of fish products contaminated by methylmercury and cadmium.

Contamination of foodstuff may be caused by using raw materials (plant/animal) with a high content of heavy metals or it can be caused during the phase of foodstuff manufacturing, packing and storing.

Let us study some specific cases:

5.10.5 Lead ^{82}Pb

- Presence everywhere
- Absorbed and gathered from plant and animal organisms
- Main sources of pollution:
  - metallurgic and ceramic industry;
  - benzenes (tetraethyl of lead and tetramethyl of lead);
  - escaping from lead containers.

Allowed limits (in foodstuff) Supplement 1 Regulation (CE) number 466/2001 of the Commission	**Maximal content (mg/kg live weight)**
Cow milk (fresh milk, milk designed for the production of dairy products and milk thermal treated according to directive 92/46/CEE	0,02
Beef, pork, lamb meat, poultry according to the article 2, letter a), directive 64/433/CEE of the Council (14), regulated by the directive 95/23/CE of the Council (15) and article 2, paragraph 1, directive 71/118/CEE of the Council (16), regulated by the directive 97/79/CE of the Council (17), exclusive of bowels according to the article 2, letter e), directive 64/433/CEE and article 2, paragraph 5, directive 71/118/CEE	0,5

5.10.6 Cadmium ^{48}Cd

- It is not the relevant (main) element ;
- It has the similar characteristics as Zn;
- Considerable effect on the environment because of its wide use in industry;
- Easily absorbed by the soil through plants;
- It gets into animals mostly through breathing in.

Strange case: content of cadmium in horses:

- In the liver the values much higher than alert limits were recorded (limits from the law or MRL are missing);
- It is partially motivated by a high number of metallotionein in liver;
- The monitoring plan – young and adult individuals – analysis of liver and muscles;

Allowed limits (foodstuff) Supplement 1 Regulation (CE) number466/2001 of the Commission	**Maximal content (mg/kg fresh weight)**
Beef, lamb, pork meat and poultry according to the definitions of article 2, letter a), directive 64/433/CEE of the Council (14), regulated by the directive 95/23/CE of the Council (15) and article 2, paragraph 1, directive 71/118/CEE of the Council (16), regulated by the directive 97/79/CE of the Council (17), exclusive of bowels according to the definitions of the article 2, letter e), directive 64/433/CEE and article 2, paragraph 5, directive 71/118/CEE	0,5
Horse meat	0,2
Beef, lamb/sheep, pork, poultry liver	0,5

Fish muscle according to the definitions of categories a), b) and e) of the list of the article 1 instruction of the Council (CE) number 104/2000, exclusive of species stated in 3.2.5.1	0,05

5.10.7 Chromium ^{24}Cr

- Toxicity in connection with valence;
- Nutrition importance Cr^{III} as a cofactor influencing the effect of insulin;
- Considerable toxicity Cr^{VI} on the level of lungs and kidneys;

The content of chromium in foodstuff of animal origin is not regulated by the norms of EU.

5.10.8 Mercury ^{80}Hg

- Presence in ecosystem in natural form or as a result of the activity in industry;
- It is an indirect contamination;
- It is absorbed in organic form (bacterial methylasa);
- Toxicity in connection with considerable bioaccumulation;
- Mercury of natural character can be found both in inorganic form (metal, univalent and bivalent) and in organic form (aryl and alkyl-derivates). Inorganic forms are toxic;
- Inorganic mercury can be transformed into organic form by microflora and microfauna found in the environment.
- The most toxic among all organic forms is methylmercury.
- Chromatographic techniques help us to distinguish between organic and inorganic mercury.
- They re not commonly used because of their complexity and costs.
- The total content of mercury is defined by means of pectrometria of atom absorption.

Allowed limits (in food) Supplement 1 Regulation (CE) number 466/2001 of the Commission	**Maximal content (mg/kg fresh weight)**
Fish products with the exception of those in 3.3.1.1	0,5
Lophius piscatorius (*Lophius* spp.), *Anarhichas lupus*, perch (*Dicentrarchus labrax*), Molva azzurra (*Molva dipterygia*), Palamita (*Sarda* spp.), eel (*Anguilla* spp.), Mustella (*Lepidocybium flavobrunneum*), Ipoglosso (*Hippoglossus hippoglossus*), Tonnetto (*Euthynnus* spp.), Aguglia imperiale (*Makaira*, spp.), Ruvetto (*Ruvettus pretiosus*), pikes (*Esox lucius*), Palamita bianca (*Orcynopsis unicolor*), Palombo (*Centroscymnes coelolepis*), skates (*Raja* spp.), Scorfano (*Sebastes marinus, S.mentella, S. viviparus*),,Pesce vela (*Istiophorus platypterus*), Pesce sciabola (*Lepidopus caudatus, Aphanopus carbo)*, sharks (all species), Tirsite (*Lepidocybium spp.,Ruvettus* spp.), Storione (*Acipenser* spp.), Pesce spada (*Xiphias gladius*), tuna (*Thunnus* spp.).	1,0

5.10.9 Mycotoxins - creation

Mycotoxins are toxic metabolites which are produced by some micromycets parasitizing on plants and/or food products during their development which may be accumulated in the concentration which can cause intoxication when consuming foodstuff by people or feedstuff by animals.

At present we know more than 300 mycotoxins and numerous types of mycets, which produce them, were recorded: *Aspergillus, Penicillum, Fusarium, Claviceps, Alternaria, Cladosporium, Rhizopus, Monilia*, etc. (Table1).

Table 1
The major toxigenic species of fungi and their principal mycotoxins

Fungal species	Mycotoxins
Aspergillus flavus; *A. parasiticus*	Aflatoxins
A. flavus	Cyclopiazonic acid
A. ochraceus; *Penicillium viridicatum*; *P. cyclopium*	Ochratoxin A
P. expansum	Patulin
Fusarium culmorum; *F. graminearum*; *F. sporotrichioides*	Deoxynivalenol
F. sporotrichioides; *F. poae*	T-2 toxin
F. sporotrichioides; *F. graminearum*; *F. poae*	Diacetoxyscirpenol
F. culmorum; *F. graminearum*; *F. sporotrichioides*	Zearalenone
F. moniliforme	Fumonisins
Acremonium coenophialum	Ergopeptine alkaloids
A. lolii	Lolitrem alkaloids
Phomopsis leptostromiformis	Phomopsins
Pithomyces chartarum	Sporidesmins

The main mechanisms of mycotoxins effects on the animals organism cause changes in utilising nutrition substances, their absorption and metabolism; besides, they cause changes of endocrine function and lowering of the immune system of the organism as well.

5.10.10 Aflatoxins

The most dangerous mycotoxins for people's health and animal's health are **aflatoxins.** They are produced by species *Aspergillus flavus* (aflatoxins B_1 and B_2) and *A. parasiticus* (aflatoxins B_1, B_2, G_1 and G_2), saprophyte micromycets, which may development both on the field and in the storehouse. The optimal temperature of he growth of mycets is approximately 25°C; their development is besides supported by relative moisture higher than 85%. Releasing aflatoxins may be manifested in both before and after phases of gathering.

They may be found in many food products such as oily seeds, cereals, dried fruits seasonings mostly when they are from tropical and subtropical countries.

Milk, dairy products and cheese may be contaminated by **aflatoxin M_1**, e.g. metabolite of hydroxilt of aflatoxin B_1, which is produced in liver and kidneys during hydroxilacia of aflatoxin B_1, to which all dairy cows are exposed through consuming nutrition – contaminated feedstuff. The portion of aflatoxin B_1 transformed by the

cow is 1-3% from the consumed amount. By the regulation number 1525/98/CE of European Commission maximal acceptable limit for aflatoxin M_1 in milk 50 ppt (0.05mg/Kg = 50 x 10^{-9} g/kg of milk) was defined.

5.10.11 Ochratoxin A

Ochratoxin A is produces mostly by *Aspergillus ochraceus* a *Penicillium verrucosum*, saprophyte mycets which can be found everywhere and cause cereals, feedstuff and food products going mouldy.

For the growth of mycets which produce ochratoxins in cereals minimal moisture 15-16% and temperature 4-37°C are necessary.

It gets into human body mostly through intake of cereals, legumen, coffee, wine and pork meat.

5.10.12 Other mycotoxins

Deoxivalenol and nivalenol (which belong to group trichotecens) are toxins which are very often found in foodstuff. They are mostly found in cereals as corn, barley, wheat; they are contaminated by some species of *Fusarium* such as *F. graminearum, F. culmorum, F. crookwellense*.

These species are important causal organisms of cereals fuzariosa, which develop mostly in areas in mild climate under the conditions of relatively high moisture and mild temperatures (10-30°C).

Toxin T-2 is highly toxic trichotecen, which is produced mostly by *Fusarium sporotrichioides*, species which has a weak lateral ability and which is slightly developed in cereals on the field. As opposed to dezoxinivalenol a nivalenol this toxin is much less spread with foodstuff and is a problem only with moist cereals left on the field in the autumn or with the winter species.

Zearalenon is mycotoxin produced by some species of *Fusarium*, such as *F. graminearum, F. culmorum and F. crookwellense*. The conditions of the area which supports the production of zearalenone are similar as the conditions supporting the synthesis of dezoxinivalenol, it means that relatively high moisture of air and temperature from 10 to 30°C. For this reason zearalenone can often be found in the same specimens contaminated by dezoxinivalenol, mostly corn and wheat.

Fumonisins create a group of toxic metabolites which were discovered recently and are produced by micromycets species of *Fusarium*, mostly *F. verticilloides* and *F. proliferatum*. Fumonizins are mycotoxins which can be mostly found on the corn and in foodstuff and feedstuff on the basis of corn all over the world. The most frequent among the fumonisins which were identified as natural contaminants of corn are fumonisin B_1 directly before fumonisins B_2 and B_3.

Mycotoxins – Limits

		Concern	Potentially Harmful Level Cattle	Swine
		ppm	ppm	ppm
DON/Vomitoxin		0.56	2.0 - 4.0	0.8 - 1.4
		0.56	1.0 - 2.0	0.6 - 1.0
Zearalenone		0.56	5.6 - 10.0 1.1 - 5.6	
		0.56	3.9 - 7.0	0.6 - 3.9
T-2 Toxin		0.25	0.7 - 1.5	0.7 - 1.5
HT-2 Toxin		0.25	1.5 - 3.0	1.5 - 3.0
Fumonisin	(cattle)	5.0	8.0 - 10.0	

Diarrhoetic Shellfish Poisoning (DSP) (biological test)

- Acid okadaico (OA)
- Dinofysitoxins (DTX)
- Leyessotoxins (YTX),
- Lepectenotoxins (PTX)
- Egliazaspiracid (AZA),

Amnesic Shellfish Poisoning (ASP)

- aciddomoico (AD) **20 mg/Kg**

Paralytic Shellfish Poisoning (PSP) (biological test)

- Gonyautoxin (GTX)
- Saxitoxin (STX),

Neosaxitoxin (neoSTX)

THE RISK COMMUNICATION
THE ALERT SYSTEM

SIXTH CHAPTER

INTRODUCTION

Problems connected with wrong risk perceiving are caused by different factors (demographic, cultural, psychological, etc.) and mostly by the trust to the institutions and/or for example to industry. We are still afraid if an institution releases general statement that it is dealing with the problem or that there is no reason to be worried and we realise that the possibilities to influence the decisions are poor. We are less afraid when the institution clearly names the risk and gives possible solutions when we face the decision in an open process. Risks management in foodstuff (for example radioactivity, dioxin, PCB, salmonella, etc.) is generally often based on the same "father-like" familiarisation (which provokes fear) and does not create the suitable atmosphere for creating the real alliance with the inhabitants in the course of problems such as environment and health which are our common riches. It is not enough to have knowledge from the technical point of view, it is necessary to see the environment and health as an opportunity which is necessary to be dealt with the people. In other words, the first thing it is necessary to focus our attention on is trust because only on this basis it is possible to start effective management of the problems of the environment and health problems: if in case of a disease or death communication is the social, cultural and biological right, in case of risk it is a very important factor of economic conditioning. Bad communication or just partial one is the ethical "debt" of management. It is much worse not to do it because we do not know what to say. Thus with every danger it is important for the risk management and communication to use scientific knowledge so as to set the objective of the approach between the risk which is perceived by the people and the risk which is assessed by the experts.

In the table conflict situations which often appear with the management of foodstuff risk factors are described (at the top at dx there is f.e. the presence of toxin botulotoxin in cans of industrial origin, at the bottom at Sx there are aflatoxins).

<table>
<tr><td rowspan="2">RISK EVALUATED BY EXPERTS</td><td colspan="2">RISKS FOUND OUT BY PEOPLE</td></tr>
<tr><td>LOW</td><td>HIGH</td></tr>
<tr><td>LOW</td><td>Absence of opposites</td><td>Opposites
Experts calm down

Representatives of people warn people and people do not take the warnings seriously</td></tr>
<tr><td>HIGH</td><td>Opposites
Experts warn people

People do not take warning into account</td><td>Agreement on priorities
Activites to lower the risk</td></tr>
</table>

This picture also marks the role of institutions supported by experts with the most effective content and the way the knowledge should be announced.
In this sense it is necessary to deepen the aspect of *Evaluation of perceiving risk* in qualitative terms (f.e. *focus group*) and in quantitative terms (f.e. research) by standardized and scientific methods.

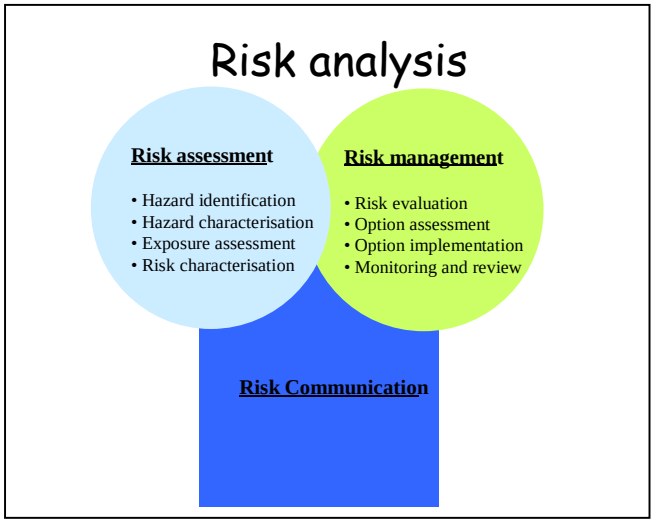
Mostly on the field of health protection against the foodstuff contamination where causal organisms are found which are dangerous for the people's health, the system of health alert is activated as it is described in the first chapter.

6.1 DEGREES OF INFORMATIVENESS

In this field three degrees if informativeness were defined:
1. Alerts
2. Information
3. News

6.1.1. Alerts:

- ***Conditions***
  - the product is on the market
  - it refers to more than one member states
  - it requires immediate steps
- ***Procedures***
  - coordination between member states of EÚ
  - taking the product off the market



6.1.2. Information:

- ***Conditions***
  - Immediate steps are not required
  - Providing useful information
- ***Procedure***

  no steps are required;

6.1.3. News

- ***Conditions***

  No condition assumed for alert or information
- ***Procedure***

no interferences are required.

SUPPLEMENT

RAPID ALERT SYSTEM FOODSTUFF AND FEED (RASFF)

ÚVOD

The White Book of foodstuff safety of the European Union from 2000 published by the European Commission assumed rigorous revision of the legislation in the field of foodstuff within member countries of the European Union. The reason for such a revision comes form the need which the changes represent which happened on the international scene from the technological, social, economic and health care point of view. Besides it was necessary to rationalise and simplify the existing legislation which seemed to be disordered and non-complex. The White Book from 2000 assumed creation of independent European institution of foodstuff safety whose role would be to assess the risk in the field of foodstuff and expand the existing system of alert with foodstuff and feedstuff, to make it more effective and exceed it to the products coming form third countries. Exceeding the system of feedstuff corresponded the most general principle according to which foodstuff safety should be carried out by means of chain connection "from farm to fork". This system corresponds with the other of the main principles which can be found in the White Book and which relates to the tracing up of the foodstuff.

RASFF

Implementation of the principles expressed in the White Book was done by means of regulation 178/2002 CE. Besides important indications of programme and operative type this instruction proves creation of "rapid alert system for foodstuff and feedstuff" (RASFF). In the articles 50, 51 and 52 regulation 178/2002 CE modalities are defined in which the new system should work.

The system which meets the demand to communicate every serious and direct risk for the health of people and which is represented by foodstuff, feedstuff or their ingredients for the purpose of their identification and taking off the market, it is organised in the form of network which common points are national contact centres designed in all member states and in the European Commission itself.

Activating the system RASFF for every danger and risk represents the phase of risk management.

In an Italian organisation the national contact centre the Department VI Directorate-General of veterinary health care and nutrition of Ministry of Health. This Department receives electronic mail signalisations coming form places of control at the customs, from veterinary authorities EÚ and authorities of marine and aviation

health care. These institutions work out data which are the subject of transmission and receive information by means of regional services, veterinary services or foodstuff hygiene of local health care units, from Experimental zooprophylactic institutes or other laboratory analyses (regional agencies of environment or supranational prevention organisations). The following ten items established in Italy which correspond with the subject of announcement in case of products coming from third (developing) countries (following five if they come from member states) undergo controls which relate to danger of the same kind.

Dataway is just the opposite, it means that from the Commission to the national contact centre and from there to the periphery in case that the risk is represented by the product which was identified in other member state.

The mechanism of rapid communication which has been increased in recent years is an important part in assessment of possible risks and in the protection of the consumer.

Dataway of the cases “alert/alarm“ must guarantee both the integrity of information and the promptness of communication.

Information is thus announced and spread among member states in the network and in real time.

The activity of the alert system assumes taking the products which are dangerous for people’s health and animals off the market. In case of serious and direct danger besides immediate detention of the products by means of intervention of competent authorities, the alert procedure may be completed by the statements in the press.

In such a case people are informed about the risk which is connected with the intake of certain product and about the way of foodstuff delivery to the local sanitary unit which is competent to act in the given area.

In case of products which cannot be found on the market of EU and for which it is not necessary to take specific measures at the side of member states, a simple report will be published with the information in order to let consumers know the risks and accepted measures also in case there are no necessary interventions at the side of the recipients of this notification.

The European Commission created a space designed for consultations on line on its web site in relation to weekly notifications sent by member states.

Web-site enables to get to know the weekly notifications which are divided into:

new alert notification for risk products which can be found on the European market.

new information notification for products which cannot be found on the European market or on which measures of control in the given state were applied.

NEW LEGISLATION OF EU IN THE FIELD OF FOODSTUFF – SANITARY CODE

Packet of hygiene introduces general norms in the field of hygiene for all foodstuff and for all operators in the field of industry.

It is represented by three hygiene codes and two instructions and two instructions for authority controls:

Hygiene codes are designed for producers while instructions about authority controls are designed for competent institutions.

Instructions come into operation on 1st January 2006.

Regulation 852/2004 EK of European Parliament and European Council for the foodstuff hygiene:

It is applied to all products and all production and distribution phases. For the first time in the legislation of EU, primary producers, farmers are considered to be in the full extent as producers of foodstuff.

It contains principles in connection with the system of rules and control "from farm to fork" according to which the most effective system of foodstuff control cannot concentrate on products but manufacturing processes and takes into account all the phases that create this process.

The degree of the primary responsibility in the field of foodstuff safety is defined for the producer. He will be obligatory to produce foodstuff and keep the plan of self-control which he will prepare and which will manage all his activities with the hygienic production of foodstuff. All the producers will be requested to work in the mode of self-control and which will be applied according to the principles of Hazard Analysis Critical Control Point (HACCP) with all the producers with the exception of those who are in the primary sector: it will be necessary for the breeders to work out only "correct hygienic practice".

The principles of HACCP note the phases which are necessary to be done when defining and managing hazardous control points. All the phases of the control must be recorded in order to document their real application.

The next criterion which was established by this regulation is the necessity of production companies to have the authorisation or at least registration with competent institutions; the registration is necessary for everybody while the authorisation will be requested with some types of productions and will be related to special procedures of authority control.

Besides it will be necessary for the producers to organise tracing up of their products; it is for the purpose of taking the product which shows to be risky off the market or for the products to be always able to show their character.

Technical supplements refer to general regulations for foodstuff production and in specific cases also products of animal and plant origin in all the phases.

Besides they dictate requirements referring to: places, conditions of transfer, facilities, waste management, water supplying, staff hygiene, pack, transformations, and formations.

Products imported from the countries outside EU must be in correspondence with the regulations of this instruction.

This instruction cancels instruction 93/43.

Instruction 853/2004 EK of the European Parliament and European Council which defines specific norms in the field of hygiene of foodstuff of animal origin

The field of application refers to all foodstuff of animal origin but specifies treatment which is carried out on the fresh meat (red meat, poultry and game), live bivalvular shellfish, fish products, fresh milk and dairy products and cheese. The role of authority vet is defined in the field of production of red meat, he carries out the activity of authority control divided into verification in the form of audit of the procedures of self-control and control of information of the chain, examination ante and post mortem, materials with specific risk laboratory tests, health marking. Specific activities of inspection staff are defined as the same in relation to the training needs which are necessary for achieving qualification and its keeping.

Instruction 183/2005 EK of the European Parliament and European Council which defines requirements of the feedstuff hygiene

The responsibility referring to feedstuff producers is the same as that of foodstuff producers. The companies from this field are necessary to register at the competent authorities and self-control is carried out according to the principles of "correct hygienic practice ".

The instruction includes specific regulations for correct practice of nutrition of animals grazing, in the stable, feeding and giving water.

Instruction 854/2004 EK of the European Parliament and European Council which defines detailed norms for the organising authority inspections of foodstuff of animal origin designed for intake by people.

This instruction is applied to foodstuff of animal origin and includes seventeen topical vertical regulations. It is not applied to the phases of distribution in retail trade and to compound products.

Facilities for the production of these products must have the authorisation with assigning the recognising number which is assigned by the competent office.

It was determined that the obligatory mutual information exchange within foodstuff chain will exist.

It describes requirements for meat production from ungulates, poultry, brood game and bag game, minced meat, meat preparation, meat mechanically sliced, meat products, bivalvular live shellfish, products from fish, milk, dairy products, eggs and other products of animal origin.

Besides it was determined that inspection activity must be based on the risk analysis.

In technological supplements the requirements are named which are assumed with regard to: places, calibration and labelling, hygiene requirements, standard temperatures and microbiological criteria for different types of products.

Seventeen vertical sector regulations are thus cancelled.

Instruction 882/2004 of the European Parliament and European Council referring to authority inspections which are aimed at the agreement with the norms in the field of feedstuff and foodstuff and norms about the health of animals

The objective of the instruction is to improve the effectiveness of authority inspections of foodstuff designed for the intake by people and enable member states to verify with regard to national regulations and regulations of EU in the field of foodstuff legislation. Seven vertical regulations are cancelled.

According to the instruction authority the inspections will be carried out on the regular basis without notice and in any phase of production, distribution of feedstuff and foodstuff. They must be based on risk analysis, previous experience and reliability of the inspections carried out by the operators themselves.

Member states set authorities competent to carry out authority inspections. These authorities must work according to the principles of neutral effectivity and effectiveness.

Therefore they must have the suitable facilities ad specifically trained and qualified staff. The correct coordination among potential central and peripheral branches of the system is necessary.

Some functions such as activities connected with audit, inspections and laboratory activity can be committed to institutions which do not belong to public authorities if they were verified at the side of member states.

Member states must prepare more than one year inspection plan which they will introduce to the Commission. The plan will contain:

•Structure, organisation, defining inspection institutions

•Targets and sources

•Potential authorisations (verification methods)

•Emergency plans

•Training the staff

LEGISLATION UNDER PREPARATION

Legislation being prepared in the field of foodstuff safety must be taken into account:

Instruction about application flexibility of HACCP in small companies

The instruction proposal assumes defining the keeping of the instruction 852/2004 recital 15 and article 5, criteria for defining the types of production companies on which the level of production reasons simplified application of self-control according to principles HACCP. It would refer to the companies which would provide the dangers control by means of simpler methodologies of HACCP by their level of production or type of products. In such ceases the proposal assumes the application of HACCP in certain foodstuff companies in a flexible way while the self-control in the course of application of correct hygiene practice is limited.

Instruction about microbiological criteria

The instruction proposal assumes defining critical limits for some dangers and for some types of products. Producers should follow these limits when applying self-control according to principles HACCP.

AUTHORITY INSPECTION OF FOODSTUFF IN ITALY

In Italy authority inspection of foodstuff is carried out by the Ministry of Health which works as a central competent authority through “Directorate of health of animals and foodstuff“. This institution has national competence in the field of national and international legislation, it carries out audit in relation to regional institutions, the inspection of customs centres in all the matters regarding the foodstuff safety and safety of veterinary sector.

On the regional level in every region there are so called Departments of health care which have the function of contact place between central office and local offices. Local authorities are represented by Prevention Departments at Local health care organisations (approximately 160 on the district level with the covering for the whole state area). In every department of prevention there is the Service of foodstuff and nutrition hygiene (SIAN) and Veterinary Service (SVET).

SIAN has competence in the field of nutrition and foodstuff hygiene in general, SVET has a competence in the field of health of animals, feedstuff and authority inspections all the foodstuff chains of animal origin by means of their functional zones: Sanitary system for animals, Hygiene of zootechnical production, Hygiene of foodstuff of animal origin.

SIAN and SVET have authorisation and inspection function, they carry out audit of self-control, sampling in all companies which directly or indirectly contribute to primary production, transformation or distribution of foodstuff and feedstuff.

The staff which takes part in continuous training plans has medical, veterinary, technical and administration education.

Control sanitary activity on the customs depends directly on the Ministry of Health- such as Customs inspection places (veterinary) and Offices of marine and air health care.

Authority laboratories are represented by Experimental zooprophylactic institutes (IZS) with 10 centres and 90 district locations for the products of animal origin and feedstuff and Regional agencies for the protection of environment (ARPA) with 20 central locations and 90 peripheral locations for other foodstuff. All these laboratories have the accreditation according to the norm ISO17025.

In above stated centres also activities of epidemiological observation and gathering of data through zones of competence, research and training are carried out (in cooperation with universities).

Risk assessment is provided by research centres IZS, ARPA, universities, Higher sanitary institute and National Advisory service for foodstuff safety.

DATAWAYS

The results of inspection and laboratory activity are sent by peripheral units to regional units and from them into the central database.

It is the duty of family practitioners and hospitals to fill the local dataway with every occurrence, if there is a suspicion or certainty in connection with pathological manifestations relating to foodstuff. These information give impulse for epidemiological research aimed at stating causes of focus point with the presence of organisations SIAN a SVET within their competence.

Results of these researches are recorded in the national database from where it is possible to get data used for foodstuff risk assessment.

RASFF IN ITALY

Alert/alarm in the input

Information travel through electronic messages.

Information come to the Ministry of Health from The office for foodstuff safety of the European Commission and distributive chains.

Ministry of Health informs regional Veterinary services and Services of hygiene of foodstuff and nutrition of regions in interest.

These regional services sort out the information in the input for the district organisation of SVET - products of animal origin and for the district organisation of SIAN in connection with other products.

Message goes to:

District clerk of SVET or SIAN of alert management in the course of consequent actions;

Responsible manager of SVET or SIAN takes notes of it.

District clerk for the management of alert cases activates all the workers on whose area the products which are the subject of the alert of notification were sent.

Local workers/clerks: in connection with distribution of above stated products, controls are carried out and emergency measures are taken which are necessary for the restrictions of the spreading of the danger. Where it is necessary, scientific-technological help IZS or ARPA and operative help of Regional service will be used in the course of crisis management.

Consequent and final measures will be taken by the local workers on the basis of instructions of the manager of district SVET or SIAN.

***District clerk for the management of crisis of alert and warnings*:** provides fulfilling which assumes alert system and where it is necessary, he coordinates relations to other district units which are possibly involved in the crisis.

In case of rejection by the suppliers of the products which are the subject of the alert or warnings or in case that given products were distributed also to the next territory, he offers necessary help to the local worker with the messages which are necessary to be sent to the other inspection services.

In the local archive he creates a book for every received communication of alert or warnings which will contain: communication, minutes from the inspections carried out by the inspection staff, communications sent to other Services, Regions and Ministry of Health and taken measures.

He creates and keeps the information archive of alarm/alert and warnings.

He communicates the results of done inspections and taken measures immediately through electronic mail to the responsible manager of district SVET or SIAN.

The responsible worker of district SVET or SIAN checks and confirms communications proposed by the district clerk which are sent to the region and to the Ministry of health.

Alert/alarm in the output

In case that local worker of district SVET or SIAN learns about the existence of direct or indirect risk for the health of people, he immediately sends this information to the district clerk for the alert cases management and district manager of SVET or SIAN.

Local manager takes emergency measures necessary for the restrictions of the spreading of the danger. Where it is necessary, scientific-technological help IZS or ARPA and operative help Regional service will be used in the course of crisis management.

Consequent and definitive measures are taken by the local manager on the basis of instructions of the responsible manager of the district SVET or SIAN. Each measure carried out in connection with the product must have as the target limitation of placing of the product on the market or intensification of taking foodstuff or feedstuff off the market.

The district clerk for crisis management in alert cases announces the taken measures and all the other known information relating to the degree of risk of the region.

The region sends the information to the National contact place for the alert cases

RASFF is consequently activated.

In case of information coming from the Office of customs control, this is sent directly to the National contact place for the cases of alert. In such a case the responsible manager of the Office of customs control takes emergency and constant measures according to the same modalities as district services.

SELF-CONTROL ACCORDING TO THE METHOD HACCP

PRESENTATION

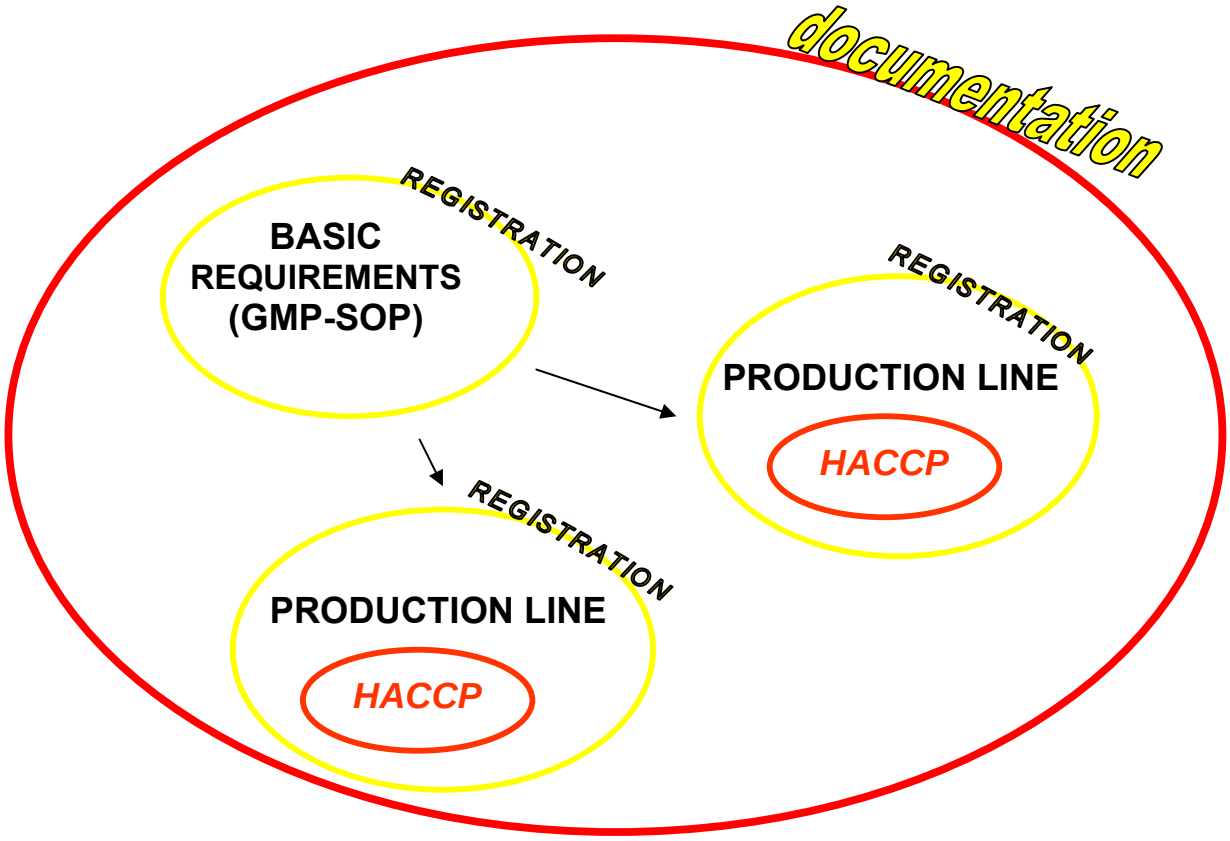
The method HACCP is a precautionary system of control aimed at the guarantee of *sanitary safety* of foodstuff by means of identification of specific dangers, their assessment and accepting precautionary procedures of control and management.

This new philosophy is based on objective and quantifiable elements and is combined with direct responsibility of the producer. It brings a turn in the system of hygienic-sanitary protection from repressive measures to the new way of preventive type.

From the experience taken from the implementation of the method HACCP with different types of foodstuff production (from bakery to vegetable cans, from slicing meat to smoked-meat products shop, from catering industry to restaurants) this proposal arose and its objective is to simplify the logical sequence of elements necessary for elaborating the manual.

The applied method is based on the clear differentiation of good manufacturing practice, which is considered to be the essential requirement of real method HACCP.

There is the division into two parts from which the first one refers to preventive requirements and the second one refers to the development of HACCP on individual production lines according to the scheme:


documentation
REGISTRATION
BASIC
REQUIREMENTS
(GMP-SOP)
REGISTRATION
PRODUCTION LINE
HACCP
REGISTRATION
PRODUCTION LINE
HACCP

LEGENDA OF THE CONTENT:

In accompanying lines the following topics are gradually developed:

CHAPTER 1 REQUIREMENTS	CHAPTER 2 PRODUCTION
DATA ABOUT THE COMPANY	DEFINITION AND DESCRIPTION OF PRODUCTS OR PRODUCTION LINES
COMPANY MANAGEMENT MANDATE	RECIPIENT IDENTIFICATION
PLAN TARGET	PRODUCTION PROCESS DESCRIPTION
GROUP HACCP	FLOW DIAGRAM
PLACES AND EQUIPMENT	VERIFICATION OF FLOW DIAGRAM
SANITATION	DANGERS ANALYSIS
UNDESIRABLE ANIMALS	DEFINING CCP
STAFF TRAINING	CRITICAL LIMITS
STAFF HYGIENE	MONITORING
PROCESSING HYGIENE	CORRECTIVE ACTIVITIES
COMMON AND EXTRA MAINTENANCE	VERIFICATIONS
WASTE DISPOSAL	DOCUMENTATION
WATER CONTROL	TAKING THE SPECIMENS
TRACING UP	
LABELING	
RECEIVING AND STORAGE OF ADDITIVES	
RECEIVING AND STORAGE OF PACKS	
RECEIVING AND STORAGE OF RAW MATERIALS	
SUPPLIERS QUALIFICATION	

PRELIMINARY ACTIVITIES OF PLAN IMPLEMENTATION

A good rule before the activation of work planning in the course of plan implementation is to interest all the experts who work in the company by means of general training for everybody and specific training for the workers on the key positions.

Everything is activated from the company management (management mandate) which using the mandate interests workers in an active way besides providing them with sources and tools also through assigning functions and responsibilities.

REQUIREMENTS

In the first chapter under the word “requirements“ a GMP (Good Manufacturing Practice) was defined that is everything a company must activate in order to create optimal conditions for processing. These procedures are general, they are aimed at places, facilities and staff, they have indirect effect on the production controlling sources of contamination and usually do not define planned monitoring with corresponding records/evidence.

The components of the procedures “requirements“ are SOP (Standard Operating Procedure) which together with GMP indirectly take part in manufacturing process and they support conditions of the environment which are suitable for foodstuff production. However, they control specific dangers by guaranteeing their inspections/management mostly on the level of risks which are difficult to manage in particular manufacturing phases. They are procedures which control operative conditions in the company including operative instructions with the control of management of process parameters.

Dangers which had already been controlled and managed through SOP would not have to be taken into consideration in the location of the danger analyses and this would contribute to simplification of the plan HACCP.

They must be carefully documented, applied and monitored in a systematic way.

The option from the point of view of the company whether to manage the process in SOP or GMP relates to the risk degree of foodstuff production.

The procedures which can be managed in SOP are as follows:

a. **Sanitation**
b. **Undesirable animals**
c. **Drinking water inspection**
d. **Waste management**
e. **Equipment and facility maintenance**
f. **Hygiene-sanitary staff inspection**
g. **Choice and verification of suppliers**

Each individual procedure must contain:

1. **target and range of application;**
2. **responsible worker and his deputy;**
3. **operative ways;**
4. **documentation.**

Operative ways will be directed by the following criteria:

a. **plan with frequency**
b. **modality**
c. **monitoring with limits**
d. **corrective activities**
e. **verifications**

In case these procedures are applied by external workers or companies, responsible worker, besides requiring entire documentation, periodical verification of keeping the agreed operative protocol is carried out as well.

METHOD HACCP

In the second chapter the attention is focused on the production and application of 7 principles of HACCP with every product or production line on the level of individual phases according to this sequence:

Supervise: *danger analyses*

Identify: *critical control points* (CCP)

Set: *critical limits*

Define: *monitoring procedures*

Define: *corrective activities*

Define: *documentation*

Set: *verification*

It is carried out by means of plan application of interventions aimed at the prevention of foodstuff danger by connecting with already accessible principles and methods such as statistic analysis of results, archiving of the documents, qualification of suppliers, process control, etc.

The programme called HACCP except for preventive definition of specific corrective activities for keeping the manufacturing process under the control, it includes the danger identification of the foodstuff production, their layout according to the hierarchy, accepting specific preventive measures of control/management of danger, definition of critical control points, e.g. stage of the process on the level on which the control is effective and economical.

In order for the application of the system HACCP to be effective and purposeful, the central element of the system is the **management mandate** without which hygiene-sanitary target stays only on the paper and will be neither shared nor respected.

If the exact mandate is missing the first elements that are influenced are the workers who are entitled to work according to customs and habits and not in the course of keeping exact operative instruction based on the science. Then practical fall in the course of working activity manifests, money and sources are invested into fulfilling norms without practical aspect.

The next aspect of primary importance is absolute authorisation by external advisor; it includes homogenisation of manuals of self-control which are similar from the point of view of production type and do not take into consideration manufacturing reality which leads to slovenly danger analysis without proper competence what creates uniformity of dangers according to manufacturing type without taking into account the history of the company or implementation of proper preventive measures of management, it can be seen on missing preliminary requirements which would keep the minimal requirements. The system of monitoring was interpreted as a system of verification, corrective activities are missing, we start from the assumption that

concerned are never wrong, documentation is not sufficient and is not elaborated according to the objective criteria which leads to total loss of preventive target.
HACCP specifies the preventive system in the course of development of quantitative type and it concentrates the economic and tool sources there where it is necessary.
The next difficulty at the side of producers is the understanding of the time need because the preventive system gives us results after a long and not short time.

The need to define the application of guidance lines sprang up from all the experience. They define, when applying principles of the Codex, those lines which are minimal requirements necessary to follow when using the self-control plan.

PRINCIPLES OF QUANTITATIVE RISK ANALYSIS OF FOODSTUFF

INTRODUCTION

Quantitative Risk Analysis (QRA) was defined as “systematic analysis of health risks connected with specific type of foodstuff, aimed at possibility estimation of undesired effect on health as a result of consumption of this product“ (Notermans and Mead, 1996).

In the last years different international organisations contributed considerably to the development of terminology and methodology which are aimed at transfer of risk analysis principles into systems of foodstuff inspection. In this context it is necessary to emphasize mostly the activity of *International Commission on Microbiological Specifications for Foods* (ICMSF) and *World Trade Organization* (WTO). Among others last guidance lines published by ICMSF accept regulations referring to the global market management from the point of view defined by WTO, applying some criteria for risk assessment.

ALOP, TLR A FSO

For this purpose the concept ALOP (Appropriate Level of Sanitary or phytosanitary protection) was established, which is also called ALR (*Acceptable Level of Risk*), defined as “level of protection which member states consider satisfactory which sets sanitary and phytosanitary measures for health protection of people and animals or plants or health within its area.” (WTO 1995).

ICMSF established this target in the criterion which is defined as TLR (*Tolerable Level of Risk*) and it is understood as “risk which the society considers tolerable in connection with other important risks of everyday life” (ICMSF 2002).

When defining TLR we take into account impact on people’s health, technological feasibility, social-economic and political impacts. This criterion can be expressed as the number of cases during a year which were caused by certain type of foodstuff risk for 100.000 inhabitants.

It is necessary to emphasise that TLR is related to social factors (and local factors) but must be set on the scientific basis.

Here are some examples of TLR, which were proposed by ICMSF:
0,5 cases of listeriosis / year for 100.000 inhabitants;

0,02 cases of cholera/ year for 100.000 inhabitants;
0,01 case of butilism / year for 100.000 inhabitants;
18 cases of salmonella / year for 100.000 inhabitants;
10 cases of hepatitis A / year for every 100.000 inhabitants.

In order for the producers/distributors/ foodstuff suppliers to transfer necessary activities on the social level into specific targets for the purpose of achieving TLR, the concept FSO was introduced (*Food Safety Objective*), which was defines as "maximal frequency and/or content of microorganisms of sanitary interest or concentration of toxins at the moment of intake" (ICMSF 2002).

FSO should be set by competent sanitary institutions; in the European Union it can be assumed that this strategic role will belong to EFSA: *European Food Safety Authority*.

We mention several examples of FSO which were proposed by ICMSF (2002): ;
The concentration of staphylococcus enterotoxins in cheese cannot exceed:
1 μg 100 g^{-1};
concentration of aflatoxins in peanuts cannot exceed 15 μg kg^{-1};
the content of *Listeria monocytogenes* in foodstuff which are not prepared for intake cannot exceed 100 ufc g^{-1} at the moment of intake;
the content of salmonella must be lower than 100 ufc kg^{-1} of powder milk.

Guidance lines of ICMSF assume the need to express the opinion on the possibility of application of FSO. Mostly if FSO for certain type of foodstuff is set for the first time, producers and sanitary institutions should confirm that this aim is technically possible to be done by means of a self-control. If FSO is possible to be done technically, the producers apply the control measures necessary for achieving criteria performance corresponding FSO. If it is not possible there are two hypotheses:
1.if it is possible, the product or process changes;
2. if it is not possible, it could be necessary to forbid the manufacture of this product.

The system of quantitative risk analysis is aimed at estimating undesirable health effects on the field of quantity because of intake of foodstuff in relation to designed FSO. This system assumes three elements: 1. *Risk assessment*; 2. *Risk management*; 3. *Risk communication*. To these three elements belong three experts who have to work in the regime of mutual cooperation and coordination: *Risk assessor* who does the estimation of undesirable effects through the scientific method, *Risk manager* who manages estimated risk and finally *Risk communicator* who sets methods and times for communication to categories in interest.

RISK ASSESSMENT PHASES

Risk assessment can be defined as “scientific assessment of undesirable health effects, known and potential ones which result from exposure of people to the risk” (FDA/USDA/CDC, 2003).

Risk assessment is done through four consecutive phases:
Danger identification;
Exposition assessment;
Defining the relation dose- response;
Risk characterisation.

Risk identification

The phase “Risk identification” assumes “identification of physical, chemical or biological causal organism or the state of foodstuff which can provoke undesirable health effect“ (FDA/USDA/CDC, 2003).

Danger identification has double origin: epidemiological statement about the examined inhabitants and statement which refers to estimation of foodstuff danger.

As for microbiological dangers if there is a suspicion on the risk manifestation connected with the presence of pathogenic microorganism in food product, it can be necessary to plan experimental research aimed at the prognosis of survival or development of microorganism in relation to the combinations of the most significant variants on microecological field (predictive microbiology). There are numerous experimental typologies which can be applied in the course of danger identification but also in the following phases of *Risk assessment* in connection with the objectives of research. The criteria of the choice of experimental approach as well as *know-how* for the planning of the experiment and for evaluation of the results are included in the part of the document which refers to the proposal for *Risk assessment*.

Exposition assessment

In the second part of risk assessment the exposition assessment is done, that is “characterisation of the source and extent of exposure of people to danger” (FDA/USDA/CDC, 2003).

Exposition assessment of microbiological risk includes the estimation of the level of microbe contamination and “competing“ of microorganisms in foodstuff at the moment of intake as well as the quality of foodstuff consumed with every meal by different groups of consumers. Information on foodstuff intake can be gained from the statistics about intakes which were published in examined geographical zones while microbiological data can be gained from inspection activity done by competent institutions or it is possible to gain them taking specimens from the food product at the moment of intake. ICMSF emphasizes the tendency to consider

analytical data as health objective which should be guaranteed at the moment of intake and not in the output from the production company.

As for dangers connected with the presence of chemical substances residues, it is necessary to carry out a quantitative estimation of organism exposure to specific causal organism which his expressed as *administered daily intake* (ADI). For this purpose it is necessary to set as a starting point *not observed effect level* (NOEL) or *not observed adverse effect level* (NOAEL) which represent the amount of xenobiotic which is not the cause of the assessment of biological effects (physiological changes such as changes in blood composition, increase of weight of some organ, considerable decrease of weight, etc.), if it is given to laboratory animals for a long period of time (during the whole biological life or its part).

Substantial interspecific changes require for the assessment NOEL to be carried out with the most sensitive animal species when researching the sensitivity against toxic effects of xenobiotics. From the legislative point of view two species are in general the most suitable ones from which does not belong to rodents (f.e. a rat or a dog); in spite of that even if we state the more sensitive one among these two species during the experiment, it need not be like this because of metabolite differences between laboratory animals and animals of zootechnical interest or because of the creation of metabolites, which cause certain toxic effects. In spite of that the procedure which is currently used for defining NOEL could lead to results which cannot always be assigned for people.
After defining NOEL for the most sensitive species this value can be transformed to ADI of animals which represents the amount of xenobiotics which may be consumed during the whole life of animal without manifestations of biological effects.

ADI of a person is reached when ADI of the animal is divided by the factor - abatement which equals 100, which results from the product of 10 (which takes into account interspecific variability) multiplied by 10 (interspecific variability).
It is assumed that a person is ten times more sensitive than species of animal on which the experiments were carried out and that within the human gender itself the sensitivity changes ten times. Despite taking into account these preventive factors, it was proved that for some compounds (xenobiotics) the variability in inter and intraspecific sensitivity is higher.

Defining the relation dose/response
In the third phase of Risk assessment *"Defining the relation dose/response"* is done defined as "defining a relation among the range of exposition and the range and/or frequency of undesirable health effects" (FDA/USDA/CDC, 2003).

In this phase the serious character and the length of duration of undesirable health effects which may manifest after consuming foodstuff in connection with the dose is estimated.

In case of microbiological danger caused by the presence of microbe casual organisms and/or products of micro metabolism (f.e. toxins) in connection with biological difference of microorganisms and their human targets under the same conditions, it is possible that there will be different manifestations with considerably different effects:

- absence of infection;
- asymtomatic infection (f.e. growth in the intestines);
- urgent pathological manifestations (not always of gastroenteric type);
- chronic pathological manifestations or long-term effects (f.e. arthritis, haemolytic uremic syndrome, neoplazia).

The variability of effects on host organism depends on different factors and mostly on:

- physiological characteristics of the microorganism;
- sensitivity of the organism;
- foodstuff characterisations (presence of barriers);
- reformation of subletal microbe damage.

Defining the relation dose/response is one of the most critical phases of the process of risk analysis and it is because of the insufficiency of experimental data which often lead to information gained from products which are responsible for diseases caused by foodstuff which were announced and this represents of course only minimal part of the total number of cases.

In this phase experimental records are gathered coming from studies realised on people and on animals which are compared with epidemiological data and with gained knowledge and experience.

Particularly referring to assessment of effect of dose/response in relation to chemical risk, it is necessary to take into account that:

- toxicity of xenobiotics is related to dose;
- toxicity assessment is done mostly by means of studies "live";
- due to interspecific variability experiments with more species of animals are necessary;
- application of toxicological data gained from laboratory animals to people despite the fact that it is disputable, at present it creates the only valid system of the assessment of toxicological risk from residues which can be found in foodstuff.

Risk characterisation

In the fourth phase of risk assessment *"Risk characterisation "* is done which represents "integration of danger analysis results, danger characterisation and exposition assessment for the purpose of reaching estimation of undesirable effects which might manifest with certain group of inhabitants" (FDA/USDA/CDC, 2003).

By risk characterisation on the basis of information gathered in the previous phases we have in mind to reach the risk estimation which is assessed as the indicator of the level of sanitary effect resulting form certain exposition (f.e. number of cases/100.000 inhabitants in one year).

In case of microbiological risk, the risk characterisation should assume confrontation between expected risk and epidemiological data and if possible we take into account the variability of the effect which can be connected with the biological diversity of stems as well as different risk distribution, different degrees of contamination and factors which influence the development and variability of reaction/response of the host.

Risk estimation is thus compared with TLR and in case that it exceeded TRL it is necessary to identify the activities aimed at control innovation.

RISK MANAGEMENT

Risk management is defined as “management of sanitary effects which are known or potentially possible and come out of danger exposure“ (FDA/USDA/CDC, 2003).

This activity can be done the same ways as with the system HACCP in connection with the activities which seven principles of Codex Alimentarius assume, but in close interconnection between *Risk communicator* and *Risk manager*. These expertnesses may dispose changes in the process and possibly activate new risk assessment in connection with modified risk/product.

In the course of management risk activities the effectiveness of the control activity may be assessed in terms of “criteria performance”, which are defined as “result of one or more control measures applied in one or more CCP for the purpose of guaranteeing foodstuff safety” (ICMSF, 2002).

In connection with microbiological danger criterion performance is usually stated as the number of logarithmic cycles, similar to the concept *Decimal Reduction Time* (D) from thermobacteriology (f.e. reduction 6D *Salmonella* spp. through thermal treatment).

Criteria performance are the result of control measures necessary to achieve specific FSO.

Some criteria performance are proposed from the scientific literature:

- Reduction 12D *Cl. botulinum* proteolitico in non-acid tins (minimum botulinum cook) (Brown, 1997);
- Reduction 6D *L. monocytogenes* in completed frozen meals (Lund et al., 1989);
- Reduction 6D psychotropic stems *Cl. botulinum* in REPFEDs (*refrigerated prepared foods with extended durability*) (Gould, 1999);
- Reduction 5D *E. coli* verocitotossico in sour/fermented products on the basis of meat (Nickelson et al., 1996).

Identification of the criterion performance represents a considerably important tool for the activities of risk management. For this purpose we have to take into account the initial danger degree but also the modification which arises during the process (lowering or increasing the danger).

Criterion performance assessed as a symbol of sum of effects which were subtracted or added to initial risk must be lower or the same as FSO:

$$\mathbf{H_0 - \sum R + \sum I \leq FSO};$$ where:

H_0 = Initial level of danger;

∑R = Symbol of sum of the effects of danger reduction;
∑I = Symbol of sum of the effects of danger increase;
FSO = Foodstuff safety object.

These two examples show the way of application of criterion performance through computation of one of the indexes of above stated equation.

The first example refers to performance of the process of the preparation of roasted meat in connection with danger which is represented by the surviving of *Escherichia coli* verocitotossico.

The data published in scientific literature show that the infectious dose for this microorganism can be lower than 100 ufc g^{-1} (AGA, 1994). Let us assume that the epidemiological data relating to certain geographical area show that the number of cases/100.000 inhabitants is increasing when the presence in foodstuff exceeds the minimal infectious dose 1 ufc g^{-1}, and consequently let us imagine that the objective of FSO is set to the level one hundred times lower: 1 ufc 100 g^{-1} (intake).

If for example during the activity of risk management in the system RASFF in frozen meat designed for the production of roasted meat, the presence of *E. coli* verocitotossico corresponding 10^3 ufc g^{-1} is found out but the growth may be prevented (∑I) because the product is frozen, the criterion performance for roasting will be expressed as follows:

$H_0 - \sum R + \sum I \leq FSO; \quad 3 - \sum R + 0 \leq -2$

CRITERION PERFORMANCE: $\sum R \leq 5$ (thus: 5D).

It means that for the purpose of guaranteeing the keeping of designed FSO in the geographical area in interest, described risk management must assume the thermal treatment at least 5D. In connection with the temperature of roasting T it will be possible to find out from the scientific literature the value D_T of the required microorganism (expressed in minutes) at the examined temperature and calculate the minimal thermal treatment which should be applied for the risk management (period/temperature).

The second example refers to performance of the process of cellaring the ham with special regard for acceptable level for the presence of *Listeria monocytogenes* in the raw material.

The data published in scientific literature show that the infectious dose for this microorganism is higher than 100 ufc g^{-1} (Buchanan, 1997). Let us assume that the epidemiological data relating to certain geographical area show that the number of cases/100.000 inhabitants is increasing when the presence in foodstuff exceeds

the minimal infectious dose 100 ufc g^{-1}, and consequently let us imagine that the objective of FSO is set to the level ten times lower: 10 ufc g^{-1} (intake).

If during the activity of risk management for example in the system RASFF we want to assess the maximal acceptable content of *L. monocytogenes* in joints, it is necessary to make an estimation for the next two indexes of equation of the criterion performance, e.g. ∑R a ∑I. We speculate that published data show that in examined conditions of the process no listeriosa effect exists (∑R = 0) and that the initial danger degree may increase during the process and distribution and thus it will equal the logarithmic cycle (∑I = 1), maximal content of *L. monocytogenes* in joints (H_0) will be:

$H_0 - \sum R + \sum I \leq FSO$; $H_0 - 0 + 1 \leq 2$;

CRITERION PERFORMANCE: $H_0 \leq 1$.

It means that for the purpose of guaranteeing the following of the objective FSO, which was set for geographical zone in interest, described risk management must assume maximal content of *L. monocytogenes* in joints of pork 10 ufc g^{-1}.

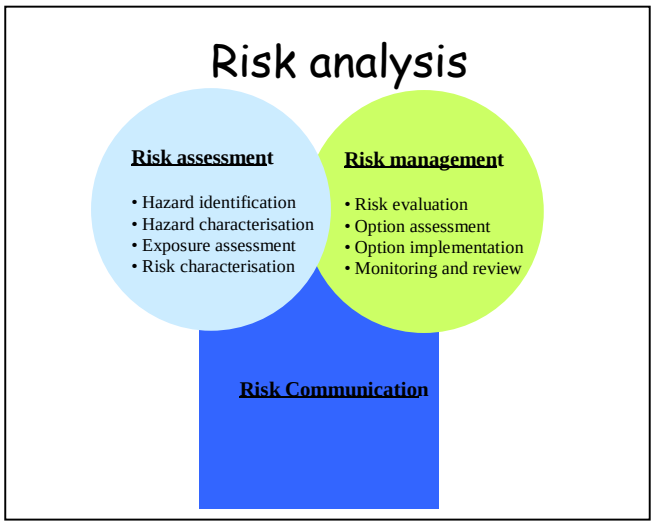
RISK COMMUNICATION

ALARM SYSTEM

DEGREES OF INFORMATIVENESS

In this field three degrees if informativeness were defined:

1. Alerts
2. Information
3. News



1. Alerts:

- ***Conditions***
  - the product is on the market
  - it refers to more than one member states
  - it requires immediate steps
- ***Procedures***

– coordination between member states of EÚ
– taking the product off the market

2. Information:

- ***Conditions***
  - Immediate steps are not required
  - Providing useful information
- ***Procedure***

  no steps are required;

3. News

- ***Conditions***

  No condition assumed for alert or information
- ***Procedure***

  no interferences are required.

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