AGRICULTURE
ENVIRONMENT

FOOD & FOOD SAFETY

INRA

200 researchers and engineers at INRA are working on food safety

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FOOD SAFETY

We expect from foods that they will provide health, well-being and pleasure. And today, we feel that an absence of any chemical or microbiological risks will be ensured. However, several recent crises or alerts (BSE, dioxin, listeriosis) have shattered the confidence of our fellow citizens. Because microbiological and chemical safety remains the prime demand from consumers. Risk prevention is an essential concern of professionals in the food industry. Any accident can result in economic loss and a major deterioration in brand image. Such prevention is based on the strict monitoring of procedures throughout the production chain, and on the existence of permanent controls performed by independent organisations. Nevertheless, technological innovations, the import of new raw materials, changes to distribution and consumption practices, and an increase in the number of high-risk consumers (the elderly, those who are immunodepressed or allergic) are constantly contributing to the emergence of new dangers. At INRA, the major challenge is to anticipate these risks in the food industry, and to develop methods for their prevention and detection in partnership with technical centres and industrialists.

Xavier Leverve
Scientific Director, Human Nutrition and Food Safety

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Research on pathogenic bacteria sometimes requires stringent safety measures.

SOME FIGURES

FRANCE (source: Institut national de veille sanitaire, 2006)

• Between 2001 and 2003, the **1656 cases of collective food poisoning** notified affected **22,113 people and caused 11 deaths**.

• **60%** of these cases of food poisoning were due to Salmonella and **65%** occurred in the context of institutional catering.

WORLD (source: World Health Organization)

• **2,1 millions adults and 3 million children** die because they have consumed contaminated water or food.

• In the USA, in 1994, an outbreak of **salmonella infection** due to contaminated ice cream affected **224,000 people**.

• In 1988, in China, **300,000 people** were affected by hepatitis A after consuming contaminated clams.

SYNERGY BETWEEN RESEARCH AND FOOD SAFETY

The mission of the Agence Française de Sécurité Sanitaire des Aliments (French Food Safety Agency, or AFSSA) is to evaluate health and nutritional benefits and risks, to recommend measures for health protection, to carry out and commission research at a national level, to ensure surveillance, watch and alerts and to provide training and information. These numerous and specific responsibilities have led us to develop a network of different actors, of which INRA is a crucial member.

In particular, our collaboration with INRA has resulted in the initiation of two Joint Research Units and the funding of joint programmes, including thesis grants.

AFSSA is an operational agency and must retain its independence from any commercial interests. On this demanding and unique position depends consumer protection and a clearer understanding of the interests of the economic sector itself.

The Agency has twelve laboratories and several entities which work in partnership with the leading specialists in France and elsewhere. In direct contact with public and private sector risk managers, as well as with consumers who can call upon it through their associations, the AFSSA identifies the needs for the knowledge or skills necessary to identify, characterise and evaluate risks and emerging phenomena, as well as ensuring implementation of its guidelines.

To evaluate risks and benefits, the AFSSA calls upon expert groups, including numerous INRA scientists. From these foundations, the agency can mobilise and initiate research at a national level. Legislation has given it the mission of coordinating (within its area of competence) all international scientific studies. This mission has seen new impetus with the creation of a European network of similar organisations, led by the European Food Safety Authority.

Pascale Briand
Director General of the AFSSA

"The knowledge acquired serves to anticipate risks"

Research at INRA on food safety is organised around three objectives. The first consists in determining why a micro-organism present in the food chain should or should not present a danger to man. Pathogenic bacteria are often similar to bacterial flora which are neutral or beneficial to man, from which they probably originate. What functions have they acquired which render them harmful to humans? What are "high-risk genes" and how do they occur during evolution? In collaboration with partners such as AFSSA or the industrial companies concerned, our research is generating more effective, practical methods for in situ detection.

The second area of work concerns the microbial ecosystems where pathogens circulate. Very numerous micro-organisms, usually inoffensive and often beneficial, surround us and colonise plants, animals and their derived foods (processed fruits and vegetables, meat or dairy products). By occupying the terrain, they often act as a barrier against pathogens and very certainly constitute our most faithful allies; these micro-organisms permit the conservation of sauerkraut, cheese and sausages, and it is also their presence in our digestive tract which educates our immune system and constitutes a barrier against pathogens. Which species comprise these populations? Can they be used as natural allies to enable the efficient storage of foods? The use of genomic data and recent developments in microscopic imaging have enabled new approaches to these questions.

A third area concerns the risks posed by residues of chemical contaminants in foods. Food toxicology is a complex affair. The compounds are ingested in a trace state in the form of mixtures. The effects of contamination which accumulate as we consume food are not immediate and the body reacts differently, which also triggers indirect effects. INRA is seeking to establish the relationship between exposure to and tissue concentrations of such residues (which vary depending on the chemical families considered), the frequency of exposure, interactions between residues and foods and each individual.

The knowledge acquired in these three research areas will serve not only to understand our "enemies" but also to model their behaviour in new situations, so that we can better anticipate the risks which we might encounter. It also provides a basis for risk analysis approaches which integrate the exposure of consumers and are being applied in partnership with AFSSA teams.

Claude Gaillardin , Head, Microbiology and the Food Chain Department.

BIOLOGICAL RESOURCE CENTRES

Research on food safety is dependent on our knowledge of pathogenic microbial agents. Identifying these micro-organisms supposes the availability of appropriate "biological materials". For this reason, in 1984, INRA initiated the collection of microbial resources: bacteria, fungi and yeasts. These collections are divided between four Biological Resource Centres (BRC) which authenticate, acquire, supply and conserve micro-organisms according to strictly limited quality parameters. Each BRC benefits from archive facilities and laboratories for biomolecular analyses which necessitate a remarkable level of security.

The BRC in Tours – which is currently being validated – will be specifically dedicated to food safety, storing some 300 strains each of *Brucella* and *Escherichia coli*, and studying the biodiversity and molecular markers of pathogenic bacteria such as *Salmonella*, *E. coli*, *Mycobacterium, Staphylococcus*, *Brucella* and *Listeria*.

The three other INRA BRC conserve filamentous fungi (Marseilles), yeasts of technological interest (Ile-de-France) and lactic acid bacteria of agrifood interest (Rennes).

Jean De Rycke, Director, Research Unit for the Study of Animal Infectious Diseases and Public Health.

GLOSSARY

Spore: When the environment because unfavourable to their survival, some bacteria form spores which contain the genome and some of the cytoplasm in a highly resistant envelope.

Biofilm: Some micro-organisms, when binding to surfaces or interfaces exposed to humid, non-sterile environments, synthesise bacterial films (a few micrometres or millimetres thick) that are difficult to "clean".

The different infectious agents:

Bacteria are natural hosts in the human digestive tract but some strains can be pathogenic. The principal families which include infectious strains or species are: *Bacillus*, *Clostridium*, *Escherichia, Campylobacter*, *Listeria*, *Salmonella*, *Staphylococcus*, etc.

Parasites include *Trichinella spiralis* and *Toxoplasma gondii*. Parasitic diseases mainly affect developing countries.

Viruses, including that of hepatitis A, are frequently transmitted via raw and contaminated shellfish, fruits and vegetables.

Prions are the agents for transmissible spongiform encephalopathies (TSE).

Natural toxins are produced by bacteria or moulds (mycotoxins) developing on foods.

THE GENOME AND ECOLOGY OF FOOD-BORNE BACTERIA

What are the genetic and environmental factors that affect the development of pathogenic bacteria in foods? To find out, we are characterising the bacteria in dairy products and studying the factors which favour their establishment in different biotopes, from milk to the consumer's intestine.

We are seeking to characterise the specific genes which allow their adaptation to different industrial (or hospital) environments.

Some bacteria are beneficial (e.g. *Lactobacillus lactis*), while others are undesirable because they impair the quality of products or even contaminate them, such as certain enterococci, streptococci and staphylococci. Laboratory studies are focusing on these micro-organisms. For example, we have observed that oxygen levels and temperature exert differing effects on the metabolism of various food or pathogenic bacteria. Thus by acting on the competition between bacteria we can control undesirable colonisations.

The experimental cheese production workshop attached to our laboratory has allowed us to monitor the dynamics between bacteria under real conditions. In addition, this is the only French cheese workshop equipped to study bacterial pathogens.

In collaboration with the AFSSA, our studies have shown that low temperatures favour the growth of Listeria, which may increase the health risk. Also, some peptides or fatty acids favour the growth of a particular strain rather than another. This work has encouraged us to study the substrates present in food products so as to reinforce or limit their availability, the aim being to ensure the safety of foods.

from a presentation by Alexandra Gruss,
Director, Lactic Acid and Opportunistic Bacteria Unit,
Jouy-en-Josas Research Centre..

THE SPECIFICITIES OF FOOD TOXICOLOGY

Jean-Pierre Cravedi
Director, Joint Research Unit
for Xenobiotics, Toulouse Research Centre

Normally, in toxicology, the effects of toxic substances are measured according to a dose/symptom gradient. A different approach is necessary in food toxicology; the doses considered being tiny and contamination being chronic and linked to the diet. Research must therefore be based on epidemiological studies which link a pathology with the consumption of a particular food. Toxicogenomics enables a correlation between a chemical substance and the regulation of one or more genes. As for metabolomics, it can establish the metabolic signature of a toxic substance. These recent approaches have opened new paths for research, notably concerning the problem of "traces" with long-term effects.

We benefit from internationally-acknowledged know-how on the fate of pollutants, mycotoxins, veterinary drugs and pesticides. This includes not only the bioavailability of these substances and their tissue distribution, but also the pathways for their biotransformation and elimination kinetics. We are also studying other, less well-known contaminants such as brominated compounds (flame retardants) or plastics.

As well as mixtures of contaminants, one of the problems of food toxicology resides in the duration of exposure, which means that it is necessary to define more sensitive windows of exposure: for example, the perinatal period. Another difficulty results from the indirect effects of contamination. An organ usually defends itself by eliminating toxic substances, but in some cases, the body itself can render a substance toxic. This is the case with the aromatic hydrocarbons produced during cooking on a barbecue which, once ingested, produce carcinogenic entities.

RISK/BENEFIT ANALYSES

Philippe Verger
Director, Research Unit
for the Methodologies of Food Risk Analysis, Paris Research Centre.

Our research unit, Mét@risk, is working on the methodologies of analysing food risks, whether they are chemical or microbiological. We are developing decision-making tools for the public sector, and evaluating the efficacy of management instruments and communication on risk. These studies focus on integrating heterogeneous data, and we have produced a software program for chronic and acute risk assessment. We can now model the impact on human health of constituents in foods according to a risk/benefit analysis. It thus becomes possible to determine what percentage of the population is at risk from a particular agent, and to characterise this high-risk population.

For example, we have recently obtained the preliminary results of a study on exposure to methylmercury linked to the consumption of tuna fish. This fatty fish is a source of proteins of excellent quality, as well as of iodine, selenium, vitamins A and D and polyunsaturated fatty acids; its consumption is recommended by nutritionists. However, as it is situated at the summit of the food chain in our oceans, the tuna is one of the fish most severely contaminated by methylmercury, a toxic compound which spreads throughout the body (notably in the brain) and can damage the nervous system of a foetus. A study of tuna consumption in France has shown that 4.4% of women of childbearing age consume large quantities of tuna fish and are thus theoretically exposed to a methylmercury overdose. These results corroborate AFSSA recommendations. We have measured the impact of communication campaigns regarding the risk related to tuna consumption: they caused a drop in consumption, particularly of tinned tuna, and differing degrees of vulnerability of industrial sectors, depending on their structure.

THE HYGIENE AND FUNCTIONALITY OF INDUSTRIAL EQUIPMENT

Thierry Benezech
Team Leader, Hygiene of Agrifood Industry Equipment, Lille Research Centre.

In the agrifood industry, cleaning operations are designed to remove all organic matter and soiling which remain on industrial surfaces, thanks to the action of fluids with thermal, mechanical or chemical effects. However, some micro-organisms have a considerable resistance to cleaning procedures.

Bacteria are known to adhere strongly to surfaces, creating biofilms made up of living or dead cells protected in a gangue. These bacteria pose a problem for industry because they colonise the areas least accessible to cleaning.

A compromise between ensuring hygiene and the functionality of equipment is that much more difficult when integrated food processing chains (which are increasingly modular and compact), comprise a series of elbows and connections between different machines. This makes it even more difficult to ensure access to internal surfaces and the action of cleaning fluids.

Our work focuses on microbial risks, starting from both ends of the problem. Firstly, we are studying the adhesion properties of spore-forming bacteria (notably *Bacillus cereus*) and the mechanisms for the development of biofilms which are resistant to hygiene procedures. And secondly, we are studying the mechanisms involved in the elimination of contaminants, so that we can model cleaning processes and thus characterise the consequences of design choices with respect to industrial food production chains. To carry out this work, we benefit from an 800 mÇ pilot workshop where we can test our hypotheses in a real situation, and a class II laboratory.

THE SAFETY OF READY-TO-EAT FOODS

Christophe N Guyen The, Director, Joint Research Unit for the Safety and Quality of Products of Plant Origin (SQPOV), Avignon Research Centre

One of the teams in the SQPOV is working on microbial risks in new, ready-to-eat foods. Agrifood innovation has led to products which increasingly contain lower levels of salt and less acidity, and which have been subjected to gentler thermal treatments so as to improve their gustatory quality while extending their shelf-lives. Control of the microbial risk is thus more and more being based on refrigeration and the choice of an appropriate shelf-life. More gentle thermal treatments (between 0 and 5°C) cannot destroy spore-forming bacteria, which are not all inhibited by cold storage.

Bacillus cereus, which is frequently incriminated in food poisoning related to cooked products, lies at the heart of our research. We are studying the diversity of this bacterium so as to better identify its virulent strains, understand their capacity for adaptation and thus better prevent the risk posed by this micro-organism as a function of the treatments applied to foods. We are also studying two key mechanisms in depth, in order to understand why B. cereus represents a risk in these new food products: its ability to adapt to cold and the production of diarrheal toxins. The entire plant sector is concerned, from the production of raw materials to the consumer.

THE CEREUS PROJECT

Initiated in January 2005 in collaboration with ACTIA and AFSSA, the Cereus project is studying the biology of Bacillus cereus, its mechanisms of adaptation to cold and the formation of biofilms. The sequencing of Bacillus cereus enables identification of the fraction of the genome which is at the origin of gene transfer between strains. Bacillus cereus federates considerable research at INRA. It is a model bacterium whose capacity for evolution has rendered it a precious "laboratory animal". The study of Bacillus cereus has thus made it possible to develop forecasting models for microbial food risks for use by the agrifood industry.

In addition, INRA sociologists are contextualising the notion of risk.

RESEARCH UNITS ON FOOD SAFETY

INRA Units and Joint Research Units with other agencies

A few European programmes

- Cascade: a network of excellence on methods to detect interferences between chemical pollutants and hormonal systems which may cause serious diseases.
- MilkbioAct: an integrated programme on bacterial flora in milk and dairy products.
- Qualicheck: an integrated programme on the origin and development of undesirable micro-organisms in the food production chain.
- TSDEinact: an innovative research project on inactivation of the agents for transmissible spongiform encephalopathies.

A few ANR programmes

- Prediction and control of the onset of markers during food processing and storage.
- Evaluation of the biodiversity and health risks related to coagulase-negative staphylococci in cheeses, cured meats and production workshops.
- Prospective study to control the emergence of new flora in foods and to refine the QPS concept.
- Metabolic and endocrine impacts of bisphenol A and diethylhexyl phtalate, food contaminants arising from plastic.
- Quantification and contribution to risk analysis of staphylococcal enterotoxins in cheeses.
- Development of transcriptome viability tests concerning the agent for Q-fever in dairy products.

A FEW RESULTS

Research

- Sequencing of the genome of the principal bacterium living on fresh meat and fish: *Lactobacillus sakei*.
- Development of a method to screen for markers of exposure to newly-formed products.
- Determination of the role of endocytosis machinery in the entry of Listeria into intestinal cells.

Valorisation

INRA holds some ten patents and licensing contracts, particularly concerning methods for the detection of pathogens and contaminants.

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