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An official journal of the International Union of Food Science and Technology

An official journal of the European Federation of Food Science and Technology

All Review-style articles in Trends in Food Science & Technology are subjected to independent peer review.

Trends in Food Science & Technology is abstracted/indexed in BIOSIS, CAB Abstracts, CABS, Current Contents (Agriculture, Biology and Environmental Sciences), Dairy Abstracts, EMBASE, Eurepsa Medica, Food Science and Technology Abstracts, Nutrition Abstracts and Reviews, Research Alert, Science Citation Index, ScieSearch. Also covered in the abstract and citation database SCOPUS®. Full text available on ScienceDirect®.
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Editorial

On 20–21 November 2007 in Brussels, the European Food Safety Authority organised a Scientific Forum as part of a series of events to mark the fifth anniversary of its inception. Entitled From Safe Food to Healthy Diets, the Forum brought together an international audience of 500 scientists and other interested parties to debate some of the more topical issues in European food safety. With over 40 countries represented, the agenda was very varied and covered some of the newer high-tech issues such as nanotechnology, intelligent food packaging and GMOs as well as more established ones such as chemical and microbiological risks and animal welfare. Participants included members of the European Commission, European Parliament, the Portuguese Presidency of the Council of Ministers, the media, consumer organisations and other NGOs, producers, food industry and the general public.

In the lively and engaging debate that took place, the media and members of the public had the opportunity to interact with scientists, industry and producers and to seek their views on food safety-related issues. In many ways, the event marked a watershed in the development of EFSA coming as it did at the organisation’s 5-year mark and, as we started the process of strategic planning for the next five years, it provided the opportunity to take on board the views of a wide range of actors in the food chain.

The Forum also allowed us to take stock of EFSA’s achievements and to debate the lessons learnt from the past. It was acknowledged that EFSA already “makes a difference” and that the fledgling Authority has delivered an impressive output of robust, transparently generated scientific evidence for risk assessors. The crucial importance of cooperation and the role of EFSA in coordinating European networks of excellence were recognised. Sharing of data and resources will become increasingly important as EFSA increases its capacity to identify emerging threats to the food supply and, with an ever increasing workload, avails of synergies with national authorities.

The importance of EFSA’s mandate in providing coherent risk communication across the EU and in ensuring that citizens received clear and meaningful communication in relation to food safety was emphasised. An integral part of the risk communication process is understanding consumer perception of risk — particularly important in the culturally diverse EU — and EFSA is constantly pushing the boundaries to increase its knowledge of this area.

Looking to the future, one of the clear messages from the Forum was that the old foes — microbiological and chemical risks — have not gone away and, in light of an increasingly globalised food chain, will continue to test our resolve. On the other hand, emerging technologies such as nanotechnology and intelligent food packaging present significant new challenges to risk assessors, not least in developing approaches and methodologies. There is increasing emphasis on the welfare of animals used in food production and Europe is at the centre of animal welfare research worldwide. EFSA will continue to play a pivotal role in developing risk assessment methodologies for animal welfare and will use its multidisciplinary, integrated working practices to address welfare where necessary. Similarly, concerns over the environment and climate change will necessitate an integrated farm-to-fork approach to risk assessment. The public health challenges of obesity and chronic lifestyle-related diseases will keep nutrition high on EFSA’s agenda well into the future and we will continue to build on the work we have already carried out in areas such as nutrient profiling, health claims and food composition.

This Special Issue presents peer-reviewed papers by speakers at the Forum who agreed to deliver a paper on the basis of their presentation. I thank all those who contributed to the Forum and in particular the authors of the papers in this Special Issue.

As we reflect on the success of the Forum, we might summarise the outcome as “much achieved, much to do”. I feel privileged to lead EFSA in its important mission.

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EFSA scientific forum  
“from safe food to healthy diets”. EU risk assessment – Past, present and future

The Scientific Forum was organised on the occasion of EFSA’s five year anniversary as an opportunity to review the first five years of EFSA’s activities and to discuss future challenges. This paper summarises the main points discussed offering a concise overview of this event and is followed by papers delivered by those speakers agreeing to deliver a manuscript based on their presentation given at the Forum. More background information, including presentations, is available on EFSA’s website (www.efsa.europa.eu).

In her welcome speech, the Executive Director, Catherine Geslain-Lanéelle reminded participants that consumer confidence in the European food safety system was low at the time when EFSA was set up in 2002. There were several food-related problems during the late 1990s, such as the BSE crisis. EFSA was established to create an independent authority and voice providing science-based advice on food safety issues to the European Commission, European Parliament and the EU Member States to support their decision-making and risk management policies.

EFSA’s scientific excellence

In his opening address, Vittorio Silano, chairman of EFSA’s Scientific Committee, pointed out that the tasks of the Authority are performed by more than 400 scientists. In addition to their regular jobs at universities, institutes and authorities all over Europe, they participate in EFSA committees, panels and working groups. The highest standards of scientific excellence, together with the most recent data and methodologies, are the absolute reference points for their work. The selection of scientists and the process of risk assessment both take place in a transparent and independent manner with declarations of interest published on EFSA’s website. When appropriate, draft guidance documents, opinions and reports undergo public consultation before being adopted.

An impressive workload delivered

EFSA scientists have delivered more than 500 risk assessment opinions, guidance documents and reports on issues such as food additives, pesticides, genetically modified organisms and biological hazards. EFSA’s current work addresses so-called “old” but still present risks, such as BSE and dioxins, as well as new and emerging risks, which include lifestyle-related risks, such as obesity. Another important challenge is the pro-active assessment of the impact that new technologies may have on the food chain, such as animal cloning, nanoparticles in foods, or active and intelligent packaging. EFSA’s scientific opinions, reports and documents are readily available on its website.

Making the difference

With its achievements and efforts aimed at scientific excellence, EFSA already “makes a difference”, stated Dagmar Roth-Berendt, MEP. Nevertheless, she called on EFSA officials and scientists to remain ambitious with regard to further developments. Roth-Berendt also stated that, in the years to come, EFSA must become the definitive food safety authority in the eyes of Member States and the international arena and that, consequently, EFSA cannot afford to demand less than the “crème de la crème” of scientists.

Representing the Portuguese Presidency, Xavier Malcata, chairman of the College of Biotechnology at the Portuguese Catholic University, saw networking and the setting of priorities as the key to maintaining scientific excellence. The sharing of knowledge and resources across Europe, as well as the effective use of Europe’s rich wealth of science, is crucial.

Speaking the consumer’s language

EFSA’s tasks include more than risk assessment and support for EU risk management policies. According to Robert Madelin, Director General for Health and Consumer Protection of the European Commission, clear and understandable communication of food safety information is also essential for gaining confidence amongst European citizens. To bridge the gap between scientific knowledge and citizens’ fears and expectations, more interaction between scientists and lay people is needed.
However, Catherine Geslain-Lanéelle emphasised that one single message was not enough to reach all of the EU’s nearly 500 million citizens. Messages need to be “culturally appropriate and meaningful and must adequately address public concerns.” One of the challenges for the risk assessor is to make advice more meaningful for risk managers. Robert Madelin concluded that the maintenance of close contacts between the EU institutions and individuals will be essential to EFSA’s success.

Today’s major challenges in food safety

Microbiological risks, chemical contamination of diet and public health issues arising from an unbalanced diet were identified as some of Europe’s major challenges with respect to safe food and healthy diet.

The burden of food-borne infections on public health remains substantial. Growing international trade has led to an increased risk of the transfer of microbes from one country to another. Such challenges require a transnational approach and new and emerging risks are regularly being identified, as a result of the continuing evolution of diseases and changing patterns of production and international trade. Like “wolves in sheep’s clothing”, microbes tend to find new ways into new food hosts.

Robust, stable and flexible public health platforms for surveillance and investigation are important for sustained progress in diminishing the risk of human infection.

Critical attention must also be paid to the environmental impact of rearing animals and growing plants.

Such issues are addressed by EFSA’s Panel on Biological Hazards. This panel provides independent scientific advice on the biological hazards related to food safety and to food-borne diseases, including food-borne zoonoses and transmissible spongiform encephalopathies (BSE/TSEs). Other issues include food microbiology and waste management issues associated with food hygiene.

Consumer perception of food safety often focuses on substances added to food. Although additives, such as sweeteners, colours and flavourings perform a desired technological function, they may have a negative effect. Therefore, their use must be subject to rigorous safety assessment based on the most recent available methods and data.

EFSA’s activities include the evaluation of many new substances and the review of specific food additives in the light of significant new scientific evidence. Additionally, developments in the field of legislation applicable to food and feed have led to a substantial increase in EFSA’s workload.

The last century has seen enormous improvements in human health, to which the amount, quality and availability of food have made a major contribution. Notwithstanding, diet and lifestyle are both important factors to consider in addressing today’s major public health concerns, such as the rise in obesity. New public health policies are being developed in Europe and EFSA is uniquely positioned to support decision-makers with the latest and most authoritative scientific advice. EFSA Opinions address issues such as the tolerable upper intake level for vitamins and minerals, Population Reference Intakes and the health effects of trans fatty acids.

The availability of choice in regard to healthy food is indispensable. Consumer choice must be supported by accurate and meaningful information on the relationship between diet and health. EFSA is currently deeply involved in providing scientific support for the Regulation on Health Claims. Key areas of involvement include providing advice on nutrient profiles for products bearing claims and the development of a guidance document for applications containing health claims, as well as assessments of their scientific basis.

Understanding attitudes towards food, nutrition and food safety

Scientific risk assessment is an essential step for consumer protection, but it must be combined with efficient communication in order to achieve consumer confidence in food. Creation of trust depends on in-depth understanding of consumer perception of food in general and of food-related risks in particular. Prof Claude Fischler, Centre National de la Recherche Scientifique — CNRS, Ecoles des Hautes Etudes en Sciences Sociales — EHESS, France, explained that, despite apparent similarities and universal features in consumer perception of risk, differences exist and remain surprisingly consistent over time. In some Member States, for example, consumers are more concerned about chemical risks, whilst in others they are more afraid of biological risks.

Striking differences also exist in Europe with regard to eating cultures. Whereas consumers in Northern European countries regard eating as an individual affair, continental and southern countries attach greater value to the social dimension of food and of sharing a meal.

Due to our consumption and “incorporation” (i.e., “taking-into-the-body”) of food, we have a special, very sensitive relation to food and to perceived food risk. The old saying of “you are what you eat” reflects this “magical” view. Research has also shown that there is a clear tendency to perceive “naturality” as superior and to consider that “denaturalisation” of food is more likely to result from the addition rather than from the extraction of components, with processing more important than content itself. Humans also tend to perceive plant products as safer than animal-derived foods, although food safety science does not necessarily confirm this view.

Claude Fischler also stressed the important differences in risk perception and ranking of risks by experts and the lay public. Risk is a probabilistic notion and whilst experts can assess and understand probability and the likelihood of risk, the most common way of thinking about risk is simply a “yes or no” view. For most, statistics and experience are hard to reconcile. An important point to keep in mind in communicating about risks to audiences who most likely
have a much more personal and immediate view, notably with respect to risks associated with food.

Combating biological risks

The spread of pathogenic food-borne microorganisms, such as Salmonella, within the food chain poses a major challenge to food safety. Researchers have undertaken several EU-wide studies to determine the prevalence of Salmonella in laying hens and in broilers, turkeys and pigs. The results support the assumption that poultry products, and table eggs in particular, are regular sources of human salmonellosis in the EU. However, they also illustrate that the prevalence of Salmonella in laying and broiler flocks, and the associated human infections, can be reduced significantly. Effective surveillance and control efforts, as implemented in several EU Member States, are the key to success.

At present, the priorities of food safety management are being shifted increasingly from measures of official compliance testing towards more goal-oriented systems. With regard to food-borne pathogens, researchers are developing models to predict the fate of pathogens along the food chain and estimate the associated health risks, in addition to testing for current contamination levels in given food samples. Quantitative microbiological risk assessment (QMRA) is a valuable tool for these modelling efforts. There is a growing demand for QMRA studies to support decision-making at the European level and EFSA is in a unique position to address this need.

The emergence of the bovine spongiform encephalopathy epidemic (BSE or “mad cow” disease) in the late 1990s was a serious challenge for the European food safety system. BSE belongs to the transmissible spongiform encephalopathies, which came into the spotlight of public interest when the BSE agent was found in 1996 to have the potential of being transmitted to humans to create a new type of the deadly Creutzfeldt–Jakob disease. Science-based control measures enacted in the EU and elsewhere succeeded in restricting the BSE epidemic to a continuous decline in recent years in most countries.

Keeping an eye on chemical risks

Consumers are exposed to a diversity of chemicals from all areas of life. Important groups of compounds are pesticides — according to a Eurobarometer survey, their residues in food are perceived by consumers as the top food safety risk — and dioxins, a group of contaminants resulting from environmental pollution.

Over 200 dioxins, dioxin-like compounds and contaminants in food are known to exist. Of great concern to consumers, there have been several incidents of contamination from dioxins or dioxin-like compounds in food and feed in the past. More than 90% of dioxin in human bodies is derived from the food chain. Several measures have been implemented, including the setting of maximum limits, the development of early warning tools, and the improvement of the analytical capacity of the EU. Monitoring programmes have indicated that these measures have been effective and that human exposure to dioxin-like compounds has decreased considerably in the past two decades. Nevertheless, the recent dioxin contamination of guar gum powder, used as a food additive, demonstrates that such vigilance will remain necessary in the future.

For pesticide residues, models exist for both acute and chronic exposure. Such models are based both on consumption data and on residue levels measured in food. These models, together with any new developments to improve them, were critically discussed during the Forum with regard to the fact that average consumption patterns do not exist in the real world. Nevertheless, further developments in methodology and analysis will lead to further improvements in food safety.

Cumulative risk assessments of chemicals sharing the same mode of action currently are being developed. A set of criteria to identify common mechanisms for a group of compounds that show a dose—response relationship has been proposed. Experience in this field already exists for triazines and chloroacetanilides, as well as for carbamate and organophosphorus compounds.

Update on food additives and flavourings

Key activities of the EFSA Panel on Food Additives and Flavourings (AFC) were highlighted. EFSA has received a request from the Commission to re-evaluate all presently authorised food additives. The AFC Panel has begun with the re-evaluation of food colours. The first evaluation, for the colour Red 2G, resulted in a negative assessment. As an example of the close interaction between risk assessment and risk management, the colour was immediately banned. EFSA’s approach to the evaluation of smoke flavours is another example of how EFSA provides the scientific foundations for the official authorisation process.

Following the publication of a new study on the potential influence of certain food colours on hyperactivity in children, the Panel is faced with another challenge in assessment and seeks additional expertise on behavioural issues when reviewing this study.

In case of large and sometimes diverging datasets, the “weight of evidence” approach is very useful. It examines the consistency of data, the robustness of dose—response and the biological plausibility of effects. This approach was for example applied to the evaluation of the food contact material bisphenol A when proposing a level for the current tolerable daily intake.

GMO risk assessment around the world

All over the world, authorities responsible for the assessment and surveillance of food derived from genetically modified organisms (GMO) have chosen different approaches to this task, but almost all are based on a common set of guidelines. This was developed by the “Task Force on Foods derived from Biotechnology” of the Codex
Alimentarius Commission, an international body jointly established by the Food and Agriculture Organisation and the World Health Organisation of the United Nations. Ongoing activities of the Codex working group ensure that the guidelines reflect the most recent scientific knowledge. According to Codex guidelines, the underlying principle for risk assessment of GMO-derived foods is to compare the GMO food with its conventional counterpart. For this purpose, the risk assessors consider any intended and unintended effects of the genetic modification and evaluate its toxicity, allergenicity and potential impacts on nutrient levels.

However, some regulators such as Health Canada, require risk assessments for any kind of product from plants, microorganisms or animals of which the heritable characteristics intentionally have been modified, regardless of the methods used. Thus, any new plant variety and not only GMO, and the foods produced therefrom, may be subject to a risk assessment.

EFSA has established rigorous rules for risk assessments of GMO and GMO-derived foods. These rules are updated continuously in the light of the most recent scientific developments. EFSA scientists currently are evaluating methods of environmental risk assessment, which includes the evaluation of the impacts of GM plants on beneficial insects.

The Australian Office of the Gene Technology Regulator has long experience with environmental risk assessment and the monitoring of environmental impacts after market release, especially with insect-resistant GM cotton. However, this experience cannot be directly applied to European agriculture because of differences in landscape and climate. Australian authorities have recognised even some positive effects of GM cotton on non-target insects, due to reduced pesticide use on GM cotton. EFSA will use post-market environmental monitoring to detect any unintended effects of GM plants. However, even the most thorough collection and evaluation of data cannot eliminate a residual uncertainty. Dealing with this residual uncertainty is a major task for the further development of risk assessment in all areas.

At the centre of animal welfare research

Animal welfare and animal health are sensitive issues involving high ethical considerations. Risk assessment methodology helps in the formation of expert opinions based on compilations of objective scientific data. Animal welfare and animal health science are made up of four main work areas — the animal itself, animal husbandry, and societal and political aspects.

To judge from the number of publications in scientific journals, Europe is at the centre of animal welfare research worldwide. Research trends reflect the need for essential understanding of animals, in order to obtain a better understanding of animal—environment interactions and, as an indicator of how animals “feel”, of the ways in which animals respond to stress.

A major field of action will be the development of a specific, standardised methodology for animal welfare. With a view to characterising risks and laying down lines for scientific research, the need also exists for the development of robust and adequate indicators, which may be direct or indirect, of animal welfare.

The research and development of risk assessment techniques for animal welfare will form part of the basis used in advising key decision-makers and other stakeholders. Future challenges in research on animal welfare and well-being will be addressed by a stronger interdisciplinary approach, the redefinition of the concept of animal welfare and the combination of the views of natural and social scientists.

With regard to the economics of animal welfare, a clearly growing willingness exists in Europe to pay the costs of welfare improvements. Combined with continued support from consumers and politicians, such willingness has facilitated the most favourable position for the further expansion of animal welfare research in over five years.

Handling new technologies — nanotechnology in food and feed

As a twenty-first century technology, nanotechnology has enormous impact in a broad variety of areas. Nanotechnology in the food chain can be used for instance during the cultivation, production, processing or packaging of food. The impact of nanotechnology on food and feed safety and on production processes is of special interest for risk asserssors worldwide.

Consumers might welcome new technologies and their benefits from nanotechnology but they also have concerns about risks for health and the environment that must be accepted and understood. “Nano is new — new is small — small is new — new is unpredictable”. This is the causal chain perceived by many individuals.

NGO representatives were of the opinion that consumers should receive information in order to make an informed choice. Communication plays an important role in the debate on new technologies. Proper communication requires facts and figures provided by science and industry. A lesson learnt in the debate on genetically modified organisms is that communication must be timely and tailored to particular target groups.

Consumer acceptance and trust will depend on the perceived benefit of innovation as well as on transparent information. Europe’s existing food law framework will serve as the basis for regulation of nano-products.

A major obstacle in this and future debates is certainly the lack of clear definitions. Industry representatives have different views towards what could fall under the term ‘nanotechnology’ or ‘nanomaterial’. It was stressed by risk managers that industry had to inform and communicate, if already authorised materials were used in a nanoparticulated form as the substance was not identical to the product that was approved already. In addition, EC representatives confirmed
that new production methods and processes are covered by the EC Novel Food Regulation.

Animal cloning — a new challenge

While still a young technology, animal cloning by somatic cell nuclear transfer (SCNT) is being publicly discussed. The birth of cloned sheep, i.e. Dolly, in early 1997 was the beginning of public debate on SCNT.

Animal cloning could be used to study genetic reprogramming, for better understanding of epigenetic changes and functional genomics, for major improvements in animal models used in medicine or in human therapeutic cloning, for gene banking for endangered breeds and species and for economically beneficial methods in agriculture.

Potential uses of animal cloning are, for example, animals with resistance to diseases (including bovine BSE — already addressed successfully in the USA — as well as mastitis, brucellosis and tropical diseases), new products with increased value (low-lactose milk, kappa-casein-rich milk, better meat from myostatin cattle), environmentally friendly animals (with lower or no greenhouse gas emissions or with low phosphate emissions), the multiplication of high producing animals adapted to given environments, and animals with potential for medical uses (xenotransplantation, pharmaceutical protein production, medical model animals).

SCNT has already been successful in a number of domesticated species. Although the overall success rate of the cloning procedure (0–20% live births, depending on species and other factors) remains relatively low, SCNT technology is evolving rapidly and the proportion of apparently healthy progeny is growing.

The composition of meat and milk or the toxicity of food and feed from animal clones or their progeny do not indicate any significant difference of products derived from sexually reproduced animals.

Animal health and welfare aspects for the surrogate dams and the clones have been investigated. Possible adverse effects on health and welfare have been identified, such as those arising from the large-offspring syndrome. In order to overcome problems of public perception and economic realities, the value of appropriately addressing public concerns cannot be overestimated. In connection with live clones, their offspring and derived products obtained from such animals, EFSA is working on a scientific opinion on food safety, animal health, animal welfare and environmental implications.

Knowing more about what we eat

The availability of reliable data on food consumption and food composition is a key tool for risk assessors working on scientifically sound intake/exposure evaluations. This is relevant when considering risk—benefit in the areas of food safety and nutrition.

Historically, there has been wide variability in the datasets collected in different European countries. This may lead to misinterpretation and less reliable results. EFSA now acts as an important centre and clearing-house for data on European food consumption. The close collaboration between EFSA, Member States and European networks and research projects will enable better harmonisation of data collection methodologies and of the available datasets thereby. Such improvements will make it possible to assess both intake of nutrients and exposure to contaminants and will contribute significantly to a sound risk—benefit assessment.

The content of nutrient profiles, i.e. the classification of food by key nutrient contents, has been used for many years by public health authorities to develop food-based dietary guidelines and related communication and labelling tools.

With a view to categorising foods as eligible or not to bear claims, the new EU Regulation on Nutrition and Health Claims proposes the establishment of a harmonised European system of nutrient profiles. EFSA will provide a sound scientific base and will support the Commission and Member States in implementing the regulation.

Active and intelligent food packaging

In the past, the key safety objective for materials in contact with foods was to be as inert as possible, i.e. to have a minimum of interaction between food and packaging. However, the development of “active” packaging requires a new approach to risk assessment and safety evaluation. The aim of active packaging is the improvement of food conservation, for example by absorbing oxygen, CO2 or liquids, or by releasing desirable substances, such as vitamins or preservatives. Intelligent packaging material gives information relevant to the history and quality of the product, such as its storage temperature or its colonisation by microorganisms.

Regulation (EC) 1935/2004 on materials and articles intended to come into contact with food provides the framework for EFSA’s safety evaluation process to help risk managers define lists of authorised substances.

At the present time, the entry of such packaging to the market is limited due to cost and acceptance issues for stakeholders in the packaging chain. Consumer acceptance and, in particular, understanding of the information provided by these new technologies will be the key to market introduction.

Analysing health risks versus health benefits

Foods may contain components that have both beneficial and detrimental effects on health. Even beneficial nutrients (usually micronutrients) can produce adverse effects if consumed in high doses or by vulnerable groups, which may be defined by such factors as age (e.g. children or elderly persons) or physiological status (e.g. pregnant women). Since the mandatory fortification of foods adopts a “one size fits all” approach, the identification of at-risk groups is crucial.
and dosage must reflect risk–benefit analysis when such fortification is considered.

Increasing the consumption of fruit and vegetables provides a good example of such complexity. Fruits and vegetables are a key nutritional recommendation, but their nutrient content needs to be taken into consideration. Nitrate appears to have some beneficial effects, such as aiding gastrointestinal immunity, but it is also metabolised into potentially harmful reactive nitrogen species, including nitrite, nitric oxide and n-nitroso compounds. Nitrite can react with haemoglobin and reduce its capacity to transport oxygen, which is particularly dangerous for infants. Nitroso compounds are carcinogens, especially for gastric cancer. Also, fruit and vegetables are not the only source of nitrate/nitrite that are consumed as it is formed naturally in the body and is present in water and cured meats. However, potential risks need to be weighed against the benefits of eating vegetables and EFSA will approach its risk evaluation in this way.

In some cases, supplementary risk–benefit assessments are necessary to provide citizens with the most balanced and best possible advice. Assessors must also consider other issues, such as product formulation and the susceptibility of consumers according to age or other characteristics. For example, there is a data gap for young children.

Communicating risks: learning for the future

In the late 1990s, crises in the food chain, such as BSE and dioxins, resulted in a lack of public confidence in the ability of public authorities to fully protect consumers against potentially unsafe food. A new EU food safety system was put in place with a “farm to fork” approach to ensure the highest level of consumer protection and a clear commitment of public authorities to provide the public with more insight and access to information on food safety issues, and, in particular, to inform citizens as early and accurately as possible in the case of emerging risks associated with foods found on the market.

Openness and transparency are key principles for both risk assessors and risk managers. With the separation of risk assessment from risk management and the setting up of EFSA, an independent, European voice on food safety issues was established. The creation of risk assessment bodies at national and European levels transformed the media landscape providing a unique point of contact and source of scientific advice on food safety issues.

Media plays an important role in informing the public about food, nutrition and food safety issues. In order to reach consumers with effective messages, scientists and science communicators will have to understand each other’s views and requirements as well as the values underlying public reactions to food issues. BBC journalist Nicola Carslaw reminded participants of media’s needs for “news” and how this may polarise or emotionalise information presented.

Communicating factually is of high importance, particularly in situations of food scares, and close co-operation between scientists and communicators is required to achieve both accurate and meaningful communications.

What is the most appropriate way of communicating with the public? Cultural differences make it impossible for a single message to reach all European consumers effectively. Core messages must be adapted to the needs of different audiences. EFSA seeks to do so through close co-ordination of messages with national food safety authorities in Member States, risk managers and dialogue with its stakeholders. The goal of these networks is to ensure that not only consistent messages are communicated, but also that they can be adapted by others taking into account the national audience and needs of target audiences.

Members of the Panel provided advice for further development of EFSA’s communications including: the need to make scientific language accessible; the importance of responsiveness in building up trust; co-operation with both natural and social scientists to inform risk communications activities and utilising the support of competent bodies at national and local levels to reach consumers with more specific, tailored messages.

Conclusions from the scientific forum

The two-day Scientific Forum brought together scientists from academia and industry and administrators from more than 40 countries. More than 500 participants attended the plenary sessions, scientific workshops and round table discussions.

Speakers and participants agreed that EFSA’s achievements during the last five years are impressive. EFSA has made major progress in contributing to EU food safety system. Through its work, EFSA has contributed to: developing high scientific standards in food safety in Europe; strengthening the food safety system in Europe through its independent scientific advice and co-operation with EU Member States; preparing for future and emerging risks and developing dialogue and partnerships with stakeholders, international partners and others.

EFSA aims to produce scientific advice to the highest standards of scientific excellence and timeliness. To do so, it needs to attract the best scientists to its Scientific Committee, Panels and Working Groups. In its five years of existence, it has succeeded in developing scientific networks enabling the sharing of knowledge, data, resources and personnel. In addition, EFSA coordinates efficient communication with Member States, the European Commission and European Parliament. This communication will be further developed within the Advisory Forum co-operation strategy.

Challenges of an increasing workload

For a variety of reasons, including emerging risks and advances in technology, EFSA’s work is continually increasing. It is crucial that EFSA has the structures and resources in place to manage this ever-growing workload to monitor its activities regularly and adjust its priorities where necessary.
EFSA will make a continuous and increasing contribution towards protecting European consumers and will maintain its vigilance against known and unknown risks. The fostering of co-operation will also help Member States considering EFSA as an important part of their own food safety systems.

Attention will be focused on new challenges associated with innovation in the food and feed sector. Emerging risks will be monitored and possible impacts assessed in collaboration with Member States.

At the end of its first five years, EFSA is now firmly established as Europe’s scientific risk assessment body in food and feed safety and animal health and welfare, nutrition, plant protection and plant health. The next five years will provide EFSA with the opportunity to definitively shape the future of both assessment and communication of food safety risks in Europe.
Building on scientific excellence via sharing of scientific expertise — The case study of food safety

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Many of the difficulties faced nowadays by society at large in maintaining a safe food supply can be effectively addressed by more and (preferably) better science. However, excellence in science will become more and more dependent on sharing of data and expertise — the nuclear idea emphasized in this viewpoint article, which conveys the major points presented and discussed by the senior author in the (invited) opening plenary lecture delivered at EFSA 5th anniversary scientific forum. Data and knowledge are usually shared within the scientific community itself — and are typically aimed at peers; however, the associated citing metrics do not often correlate with their applicability and usefulness. Funding bodies have for long recognized the value of networking and crossbreeding among scientists — in reinforcing complementarities and promoting synergies. Furthermore, these bodies have had a major effect in driving food scientists reach out of their cocooned, narrow communities toward embracing a wider spectrum of disciplines (e.g. in social and economic sciences). Web-supported databases of e.g. risk data and pathogen sequences have also revolutionized access to, and use of knowledge. Finally, a number of formal trans-European platforms have been launched by private stakeholders to promote sharing. In all cases, the thrust is to be put on trust — that what is shared is genuine and valid, and will not be subsequently adulterated or used inappropriately. Willingness to engage in free circulation of information and expertise will determine the extent of effective sharing on the long run, and of excellence of science derived therefrom — which is ultimately developed for the well-being and safety of the citizen, seen first of all as a food consumer.

Introduction

Society has for long recognized the need of science to support a safe supply of food; our scientific knowledge of the phenomena affecting food safety forms the basis of all actions that have contributed to make food supply as safe as it is today. Different countries and distinct economic areas may differ in the specific approaches to control the food supply, but they tend to act consistently in terms of stringency and efficacy of control; the trend is indeed to use science to underpin relevant decisions and global orientations. Risk Analysis has meanwhile become the framework under which the major economic zones attempt to control food production and supply. As a concept, it is well established and defined — and its component Risk Assessment has a clear, well-defined science base (CODEX ALIMENTARIUS, 2007). The use of formal risk assessment exercises to inform policy decisions and specify control criteria is, however, a relatively recent development — and certainly not yet a completely mature endeavor. However, if science is important in supporting decisions at a societal level that assure safety via legislation and control, technology is no less crucial in making safety of the food supply — which is in turn supported by science.

The technologies relevant for food safety include: those pertaining to processing, packaging and distribution — which are intended to remove (or, at least, limit) hazards in the final products; and those employed to monitor and detect hazards. Science is hereby applied both to improve current technologies and to develop novel ones — which will frequently (but not always) be introduced into the food sector after they have been tested and applied in other sectors.

Building on scientific excellence

Scientific excellence is — even in general terms, a far from consensual concept (Polt, 2006). Current measures of excellence are mostly based on accessible quantitative parameters
— e.g. number of scientific papers published, absolute impact factors and ranking of journals within subject areas, as well as statistical indicators based on the number of citations by peers. These bibliometric descriptions are undoubtedly measures of a scientist’s positioning within the scientific community, but they bear no obligatory relation to the usefulness of the underlying work. However, they are comfortable targets for scientists and handy yardsticks for funding agencies. Even the number of patents obtained by a scientist may not be a reliable measure of the applicability of his work, as the vast majority of patents are hardly ever translated into exploitable technologies — and even fewer eventually meet with commercial success. Therefore, our current concept of scientific excellence does not directly help in knowing which science best serves society, specifically in terms of food safety.

Publicly funded science — with the explicit aim of contributing knowledge to aid in control of food-borne health risks, should bear an apparent impact on food safety; otherwise, there would be a mismatch between the rationale behind risk analysis (i.e. to apply resources according to need, in order to best reduce morbidity and mortality) and the intended application of research funding. Most responsibility in assuring that the most appropriate science is done rests nowadays on policy makers — either within the most relevant funding bodies, or hierarchically above but in a position to influence them. This trend has been strengthened in recent years, as funding programs become more and more focused on generating specific items of knowledge. As part of the relevant measures pertaining to food safety taken by the European Commission (EC), successive Framework Programs (FPs) have been gradually more explicit in identifying the type of research that can (and will) be funded. This approach of narrowing specificity is carried over from assessment of applications, through periodic evaluation of the projects contracted (while funding is still active), and eventually to the ex post evaluation of the programs themselves.

Therefore, funding bodies have assumed a higher share of responsibility for the appropriateness of the science developed; in a sense, the role of commissioner (who has an idea and seeks a contractor to develop it) is gradually taking up the role of research funder (who researchers apply to with their own original ideas). On the other hand, prioritizing and goal-setting policies are somewhat inaccurate and arbitrary, and thus imperfect processes per se; nevertheless, they are required to guarantee that research funds are used in more effective ways.

It is relatively simple to establish correlations between research spending and quantitative output — but it is far more difficult to ascertain the quality of that output, in terms of impact on food safety. This difficulty arises for a number of reasons: first of all, many measures that can impinge on the incidence of food-borne diseases are not based on true science. In fact, improvement or worsening of the supposed incidence of a disease can be due to local or widespread changes in the consumer, or to erratic clinical practices devoid of reference scientific findings. The reported cases of disease are also frequently not representative of the prevailing situation — as the total number of unreported, true positive cases is usually not known. Hence, official statistics may easily be biased with regard to the prevailing situation in the whole population. Data concerning diseases associated with the effects of specific food hazards are indeed notoriously inaccurate — especially in the case of diseases that do not lead directly to death. For food-borne bacterial pathogens, the numbers quoted are typically gross underestimates — i.e. 1–10% of actual cases (Motarjemi & Käferstein, 1997), yet these diseases are normally acute and undergo a relatively rapid onset. Improvements in surveillance — possibly supported by research outputs on diagnosis and by public awareness at large, will likely lead to an increase in the number of cases eventually reported; only in such a situation will scientific output correlate significantly with recorded incidence of each food-borne disease.

Although actual values of incidence of food-borne diseases are at present often unreliable, overall trends over time might represent more useful indicators of the performance of efforts developed to address them. Pathologies caused by low level, chronic exposure to chemical hazards raise other difficulties, owing to their typical delayed onset; in such cases, one has resorted to retrospective epidemiological studies to find their causes. However, the consumer-perceived risk of developing delayed onset, uncertain-gravity diseases is considerably high (Yeung & Morris, 2001) — and the results of control measures (when and if appropriate) are expected to translate much later into a reduction in incidence of a disease.

Overall, if control measures of food safety are to be underpinned by science (as they should be), the impact of science will be hard to establish with accuracy and/or expediency; hence, it is particularly difficult nowadays to relate science production to risk reduction.

In the case of research on technologies and practices aimed at improving safety throughout the food chain, the presence of the market environment brings about a discipline that is not usually present in public health-directed science. Both intervention technologies (i.e. processes, ingredients, packaging and distribution) and detection/monitoring methodologies can be included in this category. Companies will in fact execute intramural, or alternatively contract extramural research required by searching for, developing and maintaining competitive products — while the market will ensure that only the best solutions will strive, as if there were “an invisible hand” drawn by the consumer. The research investments made by the aforementioned companies will (hopefully) be recovered afterwards via product sales: if the science supporting those products is not of a high level, then the companies will experience major constraints in obtaining legal approval for, or in competing with similar products. The aforementioned
discipline — rising from the market and impinging on science upstream, leads to an alternative concept of scientific excellence.

Therefore, the contributions to food safety from the private sector — at least those that are more technological in nature, are likely to be more efficient in terms of usefulness of research, because they are exposed to a competitive environment from the very beginning.

Sharing of scientific expertise

Many scenarios of expertise sharing have a direct impact on the efficiency of mobilization of the science community toward support of safety along the food chain. This statement appears odd, because (as discussed above) it is rather difficult to establish a convincing link between the science that is performed and the impacts on food safety that it actually brings about. However, sharing certainly assures that the right questions are asked in attempts to set priorities; it also plays an increasingly important role in guaranteeing that the best possible answers are obtained.

Many European countries maintain national and regional funding bodies, which are poorly articulated with each other — even within their own geographical and topical zones of competence. As a result, projects are often commissioned which partially (or fully) overlap. On the other hand, food safety is a broad concept, so studies relevant thereto will likely cover several distinct research disciplines — and hence will fall within the scope of different research funding bodies.

Such a lack of articulation is a general problem in research funding, and EC has addressed it in a number of ways. One initiative is the ERA-NET (European Research Area NETwork) scheme, which aims at improving cooperation between researchers, and coordination between research activities carried out at national or regional levels, in Member and Associated States. This scheme makes funds available to thematically oriented projects, which require national and regional public funders to be brought together under a common aim — for which networking is seminal (http://cordis.europa.eu/coordination/era-net.htm — July 31st, 2008). Pertinent examples are listed in Table 1, for illustrative purposes.

Another important initiative related to definition of research priorities takes the form of the European Technology Platform (ETP) program. These platforms are sector-oriented, industry-led frameworks — aimed at the definition of research and development priorities, and corresponding action plans, so as to actively contribute to future growth, competitiveness and sustainability of Europe as a whole (http://cordis.europa.eu/technology-platforms/home_en.html — July 31st, 2008). The EC has committed itself to giving a great consideration to the outputs of ETPs when designing its research calls in the 7th FP — but their impact is intended (and expected) to be much wider. More than 30 ETPs exist at present, at least four of which have a direct influence upon food safety — which are also tabulated in Table 1. These ETPs represent fora in which relevant issues are analyzed and research plans are designed — always in the context of competitiveness of the European industry, and which release proposals that are driven by all stakeholders jointly (which also aids in bringing the concept of “whole food chain” into play). The capacity of distinct stakeholder groups to articulate with policy makers in the area of food safety is considered to be of great importance, but subject to a significant uncertainty (Sargeant et al., 2007); it is clearly one of those areas in which trans-disciplinary studies are required, and new forms of communication need to be explored.

Modern science is unequivocally based on sharing (of human capital and equipments, as well as of knowledge at large). The way science is publicly funded promotes an intrinsic environment for sharing — and most collaborative projects must demonstrate sharing among partners. In the case of formal multi-centre projects, most consortia that

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<td>ERA-NET</td>
<td>Strategic plans for relevant research</td>
<td>Avoid superimposition and repetition of research efforts</td>
<td><a href="http://wwwSAFEFOODERA.net/">http://wwwSAFEFOODERA.net/</a></td>
</tr>
<tr>
<td>SCAR: Standing Committee on Agricultural Research</td>
<td>—</td>
<td>Influence on agricultural research efforts</td>
<td>Raise issues of relevance for food safety</td>
<td><a href="http://ec.europa.eu/research/agriculture/scar/index_en.cfm/">http://ec.europa.eu/research/agriculture/scar/index_en.cfm/</a></td>
</tr>
<tr>
<td>Microbiological Safety of Food Funders Group</td>
<td>—</td>
<td>Critical reviews of research, aimed at pinpointing gaps and overlaps</td>
<td>Potentiate research on microbial food-borne pathogens in UK</td>
<td><a href="http://www.food.gov.uk/science/research">http://www.food.gov.uk/science/research</a> researcherinfo/foodborneillness/microfundes/msffg/</td>
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<tr>
<td>Food for Life</td>
<td>ETP</td>
<td>Novel functional foods</td>
<td>Thematic chapter specifically on food safety</td>
<td><a href="http://etp.ciaa.eu/">http://etp.ciaa.eu/</a></td>
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<tr>
<td>FABRE</td>
<td>ETP</td>
<td>Farm animal breeding</td>
<td>Horizontal theme on food quality and safety</td>
<td><a href="http://www.fabretp.org/">http://www.fabretp.org/</a></td>
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<td>IFAHSEC</td>
<td>ETP</td>
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<td>Impacts upon safety of animal-based foods</td>
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<td>Plants for the Future</td>
<td>ETP</td>
<td>Plant-based foods</td>
<td>Effects of plant biotechnology on food safety</td>
<td><a href="http://www.epswob.org/">http://www.epswob.org/</a></td>
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* All sites accessed on July 31st, 2008.
are formed derive from, or lead to lasting (essentially informal) networks — which will exist and work beyond the time span of the project funding itself. Furthermore, academic and professional societies have a respected track record of work with food safety — selected examples are detailed in Table 2.

Such societies obviously differ in the specifics of their structure, scope and activities; however, they all employ mechanisms based on membership, academic journals, professional publications, seminars and conferences — and an ever increasing number makes also use of internet facilities to link their communities and maintain them updated.

Consequently, the overall food safety field appears to be well served by a number of professional and learned societies — dealing with essentially all aspects of this broad (and broadening) subject; as a whole, they represent invaluable resources for sharing at many levels. However, sharing is in essence dependent on trust — in the transparency and competence of partners and entities, who are responsible for exerting discipline, as well as assuring credibility, transparency and honesty. In the context of food safety, trust is usually discussed in terms of the consumer — but it should be established and maintained throughout the various communities that interact within and with the food chain (van Kleef et al., 2006).

It is widely accepted that trust requires a long time and effort to be built, but a little time and effort to be compromised. As a constitutive principle, one trusts that the data and conclusions conveyed in publications of credible bodies have been previously scrutinized — internally within the research group and externally via peer-reviewing; in this case, trust is supported by the reputation of the scientific community itself. In other cases, trust is not supported by such an ethically based, robust system — but rather by intangible, often fragile mechanisms.

Three examples of challenges in food safety — that require sharing, materialize the importance of trust. First, there is the need to share outside of classical discipline-related communities — and hence to believe in people and information that are frequently distant from one’s own area of expertise (Siegrist & Cvetkovich, 2000). Second, information and expertise — that are freely and voluntarily exchanged across the interface between industry and public agencies, are always much richer than those that are compulsorily exchanged (e.g. exposure assessments would be greatly accelerated if companies’ in-house data were made openly accessible). Third, the amount of information that is passively and actively available on internet supports raises critical problems; alarmist E-mail messages concerning spurious risks related to food lie at one end of the spectrum, but these can be quite damaging — even if they appear to the informed professional as unfounded. The non-specialist consumer may likely be tempted to believe in otherwise apparently credible language, and alter his habits accordingly. Even if (and when) the message is exposed to the recipient as a hoax, it is still possible that the consumer will either harbor some doubts as to the counter-arguments used (i.e. a putative cover-up) or distrust further messages broadcasted via the same route. In either case, trust will be damaged to some extent in the short and medium runs. Particularly relevant to the above arguments is the realization that internet is a particularly rich source of information, for both consumers and manufacturers — so the truth and transparency of the information provided should to be double-checked by default (http://www.ifc.org/newsroom/reporting/index.cfm — July 31st, 2008).

Digitally supported expression of data, coupled with computational capacity and free dissemination via the internet, have indeed revolutionized sharing in many disciplines pertaining to food safety. It is nowadays difficult to imagine quantitative risk exercises (including formal risk assessments) without probabilistic modeling software and electronically exchanged datasets: e.g. DNA sequences maintained in web-held databases constitute the grounds of epidemiological monitoring of food bacterial

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<td>SAFE: European Association for Food Safety</td>
<td>Stimulate public debate on several scientific aspects of food safety</td>
<td>Develop interdisciplinary research projects and partnerships</td>
<td><a href="http://www.safeconsortium.org/">http://www.safeconsortium.org/</a></td>
</tr>
<tr>
<td>IAFP: International Association for Food Protection</td>
<td>Provide food safety professionals worldwide with forum to exchange information on protecting food supply</td>
<td>Address food safety in various disciplines</td>
<td><a href="http://www.foodprotection.org/">http://www.foodprotection.org/</a></td>
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<tr>
<td>IFT: Institute of Food Technologists</td>
<td>Advance science and technology of food through exchange of knowledge</td>
<td>Offer: undergraduate and graduate fellowships; science-based communications; career guidance programs; and science awards</td>
<td><a href="http://www.ift.org/">http://www.ift.org/</a></td>
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<tr>
<td>IFST: Institute of Food Science and Technology</td>
<td>Professionally qualify food professionals in Europe</td>
<td>Bring together academia, government and industry</td>
<td><a href="http://www.ifst.org/">http://www.ifst.org/</a></td>
</tr>
<tr>
<td>EFFoST: European Federation of Food Science and Technology</td>
<td>Enhance interaction among food science and technology societies</td>
<td>Maintain collaborative network of research organizations within European food industry</td>
<td><a href="http://www.effost.org/">http://www.effost.org/</a></td>
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* All sites accessed on July 31st, 2008.
contaminants. Virtual tools based on mathematical models have also been developed to simulate dynamic, food safety-related phenomena in foods. The most common are dedicated to predicting the behavior of microbial pathogens in foods — although the approach also permits prediction of migration of compounds from food-contact packaging materials into the food matrices themselves.

Conclusions

Science is critical to assure a safe food supply, so efforts have been made worldwide to ensure that the most appropriate questions are being asked to and answered by the science community — in an increasingly more participated way. However, scientific excellence is not a direct measure of usefulness with respect to food safety; and uncertainties, coupled with lack of representativeness of data often hamper establishment of satisfactory relationships between generation of knowledge and reduction of risk. On the other hand, research priorities in the food area have been set in a more transparent and open manner — not only in geopolitical terms, but also in scientific scope; however, improved strategies and novel means of networking between stakeholders and policy makers are urged.

To foster sharing within classical applied science, and with interfacial social science communities, trust must be built up and preserved — and preferably include volunteer sharing of data pertaining to food and relevant to public health. Although learned societies and professional bodies — which are flourishing in the traditional scientific areas pertaining to food safety, have trust inbuilt into their nature, the same cannot be said of the new wave of sharing fora based on the internet. As a whole, new information technologies represent a genuine revolution as enabling tools — the most respected and successful of which have already addressed the question of trust in their strategies. One way or another, those fora used by the food safety science communities have managed to contribute to making the world smaller — and in doing so, they are making the food supply safer and safer.

References


Communicating risks linked to food — the media’s role

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The article draws on the experience of journalists, largely in the UK, to provide an overview of the media landscape regarding food safety. The spotlight shines on the print and electronic media coverage of risk, including how editors select stories and develop content, level of detail and tone. There is exploration of “scare stories” and explanation of why some issues are afforded more weight and space than others. The text highlights examples of news reports, mainly from the UK media, about food safety and the measures that communicators can take to ensure risk-related issues are reported clearly and proportionately. The paper touches on trust, the interpretation of data and the tensions between journalistic values and scientists.

Introduction

In the context of the EFSA Scientific Forum, “From Safe Food to Healthy Diets,” held in Brussels on 20–21 November 2007, a parallel session was organised dedicated to risk communications. It was called: “Communicating Risks: learning for the future.” The author was asked to introduce her perspective to open the session and she is pleased to share her views in this paper.

The mainstream media have a vital part to play in conveying the level of risks related to food. The job of impartial journalists, as interpreted from the BBC Editorial Guidelines (2005), is to look at the evidence, put it to the test, filter out the most relevant aspects of the findings and deliver them in digestible chunks to wider audiences. This “filtering” may result in lurid headlines and scientists complaining to journalists that the story has been misinterpreted and blown out of all proportion (Dixon, 2001). So, what is the role and perspective of the mainstream journalist when covering stories about risks linked to food?

Press and broadcast journalists, while often intelligent, inquiring and sceptical, are not necessarily academic scientists. Just like newspaper readers and TV and Radio audiences, journalists come from a wide range of backgrounds and need to appeal to their markets — a diverse range of audiences. This means that there are often tensions between the media and those trying to convey science-based risks proportionately. The motives of scientists are different from those of the media; media maxims are not the same as those of a food safety authority, environment agency or food industry researcher. Scientists may despair of media reporting and journalists’ desire for simplicity and certainty where there is none. But for responsible, clear and accurate reporting of risks linked to food it is vital that communicators understand media priorities and work with them — rather than try to fight against them.

The media landscape — a potted history

In 1996, the European media were in full cry over BSE and CJD. There were deadly outbreaks of food poisonings; acute contamination of foods by dioxins and PCBs, and then terrifying warnings about genetically modified “Frankenstein foods.” There has not been one big, similarly all-pervading food scare since, but, to fill the void, amid a chattering class quest for provenance and a stampede towards farmers’ markets and organic foods, there has been an endless frenzy of smaller-scale scares. These have included headlines about benzene in fizzy drinks, dioxins in salmon, acrylamide in crisps, pork DNA in chicken, cancer-causing E-numbers, additives linked to child hyperactivity as well as doubts about the health risks of exposure to pesticides, artificial sweeteners, coffee, red wine, salmonella in chocolate bars and lately bacon, sausages and salami.

Why does food make news?

In health-obsessed wealthy populations, particularly in the UK, where food is linked to health as never before, it is little wonder that food scares grab headlines. As newspaper colleagues sometimes put it: “The British press have a mantra of Peers, Fears and Tears.” You run a story if it is relevant to the lives of your readers and your peers, it plays to their fears and is emotive. Press colleagues argue
that the newspaper’s role is to convey issues in an accessible way, trying to get answers to the questions that their readers want answered. “If this means confronting a fear and then deconstructing it so that by the end of the article the fear is either laid to rest or heightened, then so be it.”

So, the more “everyday” the food, the bigger the headline about its danger to our health. No scare no story. The principle driver steering this is commercial. Newspapers have to sell; there is no point writing a story if no one is going to want to read it.

On a practical note, it is worth mentioning that if a food scare story breaks on a quiet news day or at a weekend it is more likely to receive more press attention and achieve greater media prominence than it may deserve.

Consumers and the media — an evolutionary relationship

When Eurobarometer (EFSAs, 2006a, 2006b) asked consumers how they reacted to the last story they had heard about a type of food being unsafe or bad for their health their responses revealed that in the UK 47 per cent disregarded what they heard in the media, or else they worried and did nothing. This was higher than the EU average of 40 per cent.

Maybe there is a link with the way the media operates in Britain compared with the rest of Europe. As a colleague on a leading UK broadsheet says: “I think the European news media is far less hysterical about stories relating to food and risk than the UK media.” She goes on: “But it is, therefore, more dull. There is huge competitiveness between the British media — which is unique in the world. This intense rivalry means a drive to rigorous investigation, thorough questioning of motives and a far more entertaining media.”

However, the notion that the UK media is more competitive and more rigorous than anywhere else in Europe is beginning to sound outdated: even cursory glances at headlines across the EU press suggest that the oft-described “British media aggressiveness” is spreading across the continent.

It may be unpalatable as a concept, but the fact is that a scare sells — and the scarier and more relevant it is the better. Perhaps one of the consequences of this, though, is a shoulder-shrugging attitude among some consumers that amounts to “food scare fatigue.” People have become bored with it, and risk than the UK media."

The weight of response or the balance of an opinion from the media actively seek out the views of their audiences. Reporters have their email address at the bottom of their articles and columns. Newspapers’ websites invite comments. Radio phone-ins and all the media’s Have Your Say message boards are inundated with audience responses. The weight of response or the balance of an opinion from audiences is then reflected in the news reports. The “citizen commentator” is becoming increasingly powerful. A publication on October 31, 2007, by the World Cancer Research Fund concluded that putting on weight, alcohol and a range of everyday foods including bacon, salami and red meat cause cancers. Part of its advice to consumers was to eat no more than 500 g of cooked red meat per week. The initial reporting of the study was straightforward. It prompted a predictable backlash from the food industry but also criticism from some other doctors, who called the advice too dogmatic and said that no one would heed it. Then the tone of the coverage changed to reflect the consumer reaction to the study. “So what IS safe to eat?” asked the UK’s Daily Mail. The interactive bandwagon had its own momentum and Radio and TV bulletins and website message boards were full of vox pops and comments such as: “I won’t be told what to eat and what not to eat. It seems that nothing is safe so I’m just going to eat anything I want.” (www.bbc.co.uk/news).

The media acts as a hall of mirrors, reflecting from a variety of angles the research, the story and the debate it provokes.

Interpreting consumer attitudes

Recent analysis of health stories in the news in the UK made uncomfortable reading for news editors with a public service remit. In their research in the UK, Harrabin, Coote, and Alle (2003) found a disparity between journalistic values and what the statistics say and that this could lead to events being presented out of proportion. Press and media reporting can prompt knee-jerk reaction from politicians who throw a disproportionate amount of money at a “risk” that has been given disproportionate prominence in the news.

If you take train crashes as an example, you find that politicians, urged on by the media, spend £10 million on safety measures to save each life — when a death on the road can be prevented for one hundredth of the cost. Yet, the danger of going out on the road, coupled with media-hyped fears over stranger danger, keep children indoors where they get fat and unfit and end up costing health services billions in bills to treat obesity later on.

Surveys suggest (Starr, 1969) that consumers are not so worried about risks associated with their own behaviour or practices (voluntary risks) as they are about risks perceived to be out of their hands (involuntary risks). While in the Eurobarometer survey (2006) obesity is mentioned spontaneously as a possible risk associated with food (albeit by few consumers), few appear to be worried about putting on weight themselves.

So why is obesity constantly in the news if consumers don’t see it as relevant to them? One UK consumer journalist puts it like this: “In my view, it is a topic that illustrates how the media does report in the public interest, rather than just to make headlines and win readers. The spectre of half the population being obese is appalling; the fact that they are morbidly overweight because they voluntarily eat too
much and don’t exercise is shocking. There is a visible cause and effect and it is hard to shut our eyes to a crisis happening around us now.” In addition, the medical profession is a powerful lobby and they have persuaded governments of the risks and costs associated with obesity, so dealing with the issue has become political, adding to its news value. The journalist adds: “Another simple explanation for why obesity will continue to be a hot topic is that the middle class media perceive obesity as a working class problem and so we feel we can get away with preaching about it.”

Trust and relationships

BSE and the other food crises of the 1990s were a catalyst for change. Consumers’ interests were perceived to have come a poor third to the commercial needs of farmers and the food industry and the self-interest of government and politicians. The lack of transparency about the risks and the uncertainty of the science exacerbated the crises.

The advent of the UK Food Standards Agency in 2000 transformed the media landscape in that country. At last journalists covering UK food issues had a welcome point of contact and reference, believing it to be as independent as any organisation of its kind could be. Yet, those same journalists retained an instinctive scepticism towards a body set up by the government. In general the UK media believe they have benefited from the FSA, just as journalists in France think they have from the AFSSA. Journalists who specialise in food and consumer affairs also say they have benefited from the European Food Safety Authority’s willingness to explain the science, publish findings on a website and have open board meetings. As a result, members of the public who become aware of EFSA are able to access decision-making processes and make up their own minds about risk levels.

Reporters tend, instinctively, to treat stories as more valid if they originate from an established consumer group or an eminent scientific body, as opposed to treating with greater caution and scepticism a story offered by a commercial enterprise perceived to have vested interests. But some journalists are certainly critical of the safety agencies for not always being quick enough off the mark. They think that there should be an immediate ban on artificial additives in children’s food, for instance.

At least one UK journalist says: “Many editors don’t yet understand the role of EFSA and see it as a talking shop, so it hasn’t made the same impact as the FSA. EFSA suffers because of much of the UK media’s innate mistrust of anything EU and made up of so-called Brussels bureaucrats.”

The media as scaremongers?

In newsrooms of the quality media, reporters are urged to think very carefully about the use of words such as “deadly,” “scare” and “panic.” But undoubtedly, some programme and news editors need to be cautious and not fall into the temptation of following another newspaper’s sexy headline that doesn’t stand up to closer editorial scrutiny.

Consider the issue of genetically modified foods. Why so much fear? There have been no proven health risks and yet the media seized on the doom-laden statements and claims of anti-GM campaigners and translated those into vivid stories. A tabloid journalist confides that he, personally, was not bothered by the prospect of genetically modified foods, provided there were effective controls in place. However, there was an appetite among sub-editors on his paper to run scare stories about GMOs and so to a certain extent he “wrote to order.” The epithet, Frankenfood, Foods, struck a chord; the paper took an editorial policy line to the effect that: “We fear the unknown and so GM food must be a danger.” Once that happens, it is hard for the pro GM industry to reverse their fortunes. However, as seasoned communications professionals argue, such a turnaround is not impossible, especially if a relevant health benefit is subsequently revealed.

The way the media portrayed this whole long-running saga does not necessarily cover journalists in glory, but is it not fair to say that the biotech industry at the time failed to communicate its case? The media were being bombarded by the anti-GM lobby, the press were given tip-offs about photogenic stunt protests while the biotech industry remained passive, ineffectual and unpersuasive; often it was evasive and perceived as secretive; its inaction allowed campaigners to get in first and take the lead from the outset.

One newspaper colleague put it rather graphically: “Scientists were beavering away manipulating our food without our knowledge and permission. No one — and especially not the media — like to feel excluded and we were reacting to the way our rights to eat the sort of food we wanted were being eroded by a powerful, secretive scientific sect.”

Concluding thoughts

Undoubtedly the authorities’ obligation and commitment to be open and accessible has made reporting food scares easier. By the same token, early release and coverage of research can spark consumer fears and/or expectations prematurely. It may well turn out that subsequent findings prove that initial fears were overstated but by then the damage has been done and a food is damned by association.

BBC editorial guidelines state: “We should report statistics and risks in context, taking care not to worry the audience unduly, especially about health or crime.” The guidelines also say: “We should consider the emotional impact pictures and personal testimony can have on perceptions of risk when not supported by the balance of argument.”

For public service journalists it may sometimes mean swimming against the media tide — going against news instinct to further public understanding. But, if no scare equals no story, journalists will not want their report to be so watered-down that their editors will not publish it.

Mostly in the mainstream press and broadcast media the mid-way happens: the headlines grab your attention and the
body of the text puts the risks in proportion, which most media practitioners think seems fair enough. But there are undoubtedly major challenges ahead for the European media in the expanding EU. With globalisation and fatter, ageing populations to report on, in spite of best intentions the media is likely to continue to focus on food as a cure-all solution or a kill-all threat.

Disclaimer
Practitioners whose views and experiences were sought during the research for this paper gave their comments on condition of anonymity.

Acknowledgements
The author would like to thank the chair of the session, “Communicating risks: learning for the future”: Professor Andrew Chesson, and EFSA for organising this event, providing the occasion to debate the key communications challenges in food safety with the other participants in this session. The panel comprised: Ragnar Löfstedt (King’s Centre for Risk Management, London); Oskar Wawschinek (Austrian Agency for Health and Food Safety); Philip Tod (DG Health and Consumer Protection); Anne-Laure Gassin (European Food Safety Authority).

References

Further reading
Consumer correspondents and health/science affairs journalists in the UK and European press and broadcast media.
European Food Information Council.
Real burden and potential risks from foodborne infections: the value of multi-jurisdictional collaborations

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The burden of foodborne infections on the public health is substantial. Many different microbes can contaminate foods and cause illness in the consumer. New and emerging problems are identified with regularity, as a result of microbial change, of changing patterns of production and trade, and the complex ecologies in which we produce and process the foods we eat. In the United States, the zoonotic pathogens Salmonella, Campylobacter, Shiga toxin-producing Escherichia coli (such as E. coli O157:H7), Listeria and Toxoplasma are major challenges. Public health surveillance is vital to estimate the burden, to detect outbreaks, and to track trends in the illness as interventions take place. Cases reported to public health authorities represent only a small fraction of those that occur, but if that fraction is known, the total number of cases can be estimated. Most cases are sporadic, occurring outside of recognized outbreaks. With systematic molecular methods for surveillance, large and dispersed outbreaks can be identified. Investigation of outbreaks of illnesses can detect new pathogens, establish new food vehicles, and identify likely points of contamination. The results of surveillance, of research based on sporadic cases, and of outbreak investigations can guide the important multi-disciplinary research in food science, veterinary and ecological sectors that are critical to improving prevention upstream from the consumer. Because microbes travel freely across boundaries, this increasingly requires a transnational approach. We can anticipate more unexpected challenges, even as current challenges are controlled. Robust, stable and flexible public health platforms for surveillance, for investigation, and for supporting interdisciplinary research that improves prevention are important to sustained progress in the field of food safety.

In the United States, the burden of foodborne infections has been estimated to be 76 million acute illnesses, 323,000 hospitalizations, and 5000 deaths each year (Mead et al., 1999). This estimate incorporates extrapolations from surveillance data for known pathogens, and a factor that provides an estimate for illnesses caused by pathogens that have yet to be discovered or clearly linked to foods. It does not measure the burden of the chronic and recurrent sequela that can follow some infections, such as the Guillain–Barré syndrome that can follow Campylobacter infection (van Koningsveld, van Doorn, Schmitz, Ang, & van der Meche, 2000) or the end stage renal failure that be late sequel to infections with Shiga toxin-producing E. coli (Garg et al., 2003), and it does not translate directly into Disability Adjusted Life Years. One of the challenges of making such an estimate is that foodborne infections are a moving target, as we continue to add to the list of pathogens, implicated food vehicles and mechanisms of contamination that affect the safety of our food.

New pathogens, and new variants of pathogens continue to be identified, and well-established pathogens may be linked to foods for the first time (Tauxe, 2002). A new serotype of Salmonella has emerged in Europe and North America, that appears to be derived from Salmonella Typhimurium, but has lost one of the two flagellar antigens (Echeita, Herrera, & Usera, 2001). This serotype, I 4,[5],12:i:- has become one of the top 10 most commonly identified serotypes in the United States, though its reservoir has not been identified (CDC, 2007d). In Bangladesh, the Nipah virus, which causes a fatal encephalitis, was recently linked to the consumption of fresh sugar palm

* Adapted from a presentation given at the Scientific Forum to celebrate the 5th year of the European Food Safety Agency, Brussels, Belgium, November 20, 2007.
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and where systematic control measures need to be applied. More attention on the low moisture processed foods, where water courses and produce fields. We also need to focus and the complex ecologies that link wildlife, pastures, involved in how and where they are grown and harvested the food safety issues of fresh produce, including the risks of much food safety efforts. We need to learn more about safety of meat, poultry, eggs and dairy, the traditional focus on the importance of complex local ecologies in fields and in produce sources of foodborne infections may be and the critical importance in the long run, these outbreaks signal the need pathogens is isolated from the implicated food. Even more rapid epidemiological detection and investigation can lead to recall of a product, even before the detection and investigating them required the collaboration of authorities across many States and jurisdictions. Rapid epidemiological detection and investigation can lead to recall of a product, even before the pathogen is isolated from the implicated food. Even more important in the long run, these outbreaks signal the need for further efforts to understand the sources of the risk, and the mechanisms of contamination. A multi-disciplinary approach may be critical to understanding the contributing microbiological, technological, sociological, and ecological factors that lead to an outbreak. To make further progress in preventing foodborne infections, we must go beyond the safety of meat, poultry, eggs and dairy, the traditional focus of much food safety efforts. We need to learn more about the food safety issues of fresh produce, including the risks involved in how and where they are grown and harvested and the complex ecologies that link wildlife, pastures, water courses and produce fields. We also need to focus more attention on the low moisture processed foods, where the contamination may occur in the factory environment, and where systematic control measures need to be applied. The multi-state nature of these outbreaks also illustrates a general point: they were detected and investigated though the collaborative networks for public health surveillance and investigation that we routinely use in the United States. These networks have been increased and strengthened in the last decade, like the Enter-net surveillance network in Europe. I hope that the exchange of methods and best practices between the various networks is of benefit to all. It is critical to understand that the nature of the outbreaks detected depends to a great extent on the nature of the surveillance that is conducted.

Public health surveillance
Surveillance is the systematic and timely collection of existing clinical or laboratory data about the health conditions in a defined population. It is a routine activity in health departments that can define the burden and magnitude of a condition that we hope to control or prevent. It can identify possible outbreaks as sudden increases of the number of cases above what is expected, triggering investigations into the exposures that the affected individuals have in common. Identifying and investigating outbreaks can lead to the source, can define immediate control and long-term prevention measures, and can stimulate scientific inquiry into new problems that are identified. Ongoing surveillance is an important tool with which we can measure the impact of disease prevention efforts. It is not research, and the outbreak investigations conducted for public health purposes often do not yield all the information that risk assessors desire. However, surveillance can generate the hypotheses and provide a platform for researching them.

We conduct surveillance of foodborne infections in the United States using several networks, each of which has been strengthened since the mid-1990s. The first is routine national surveillance in all jurisdictions that is based on clinical reports of disease. The list of infections that are nationally notifiable has expanded to include *Listeria, E. coli* O157:H7 and other Shiga toxin-producing *E. coli* (STEC), and most recently *Vibrio* infections. This reporting is a routine local public health activity, and can trigger local public health actions.

This national surveillance was greatly strengthened for *Salmonella* in the 1960s by the addition of *Salmonella* serotyping (CDC, 1965). This provided a new role for the public health laboratories in each state, which began serotyping each clinical isolate of *Salmonella*, in order to provide the epidemiologists with information about which infections might be related infections. Beginning in 1996, PulseNet, the national network for molecular subtyping of foodborne pathogens, introduced standard molecular subtyping to the state public health laboratories, collecting and comparing the resulting pulsed-field gel electrophoresis (PFGE) patterns, and identifying possible clusters for the epidemiologists to investigate. This PFGE pattern network reached full national participation in 2001 for *E. coli* O157:H7, and is now extending to include routine subtyping of listeriosis, several serotypes of *Salmonella,*
is reasonably, though not perfectly representative of the population of the United States (www.cdc.gov/foodnet). It was 41.5 million under surveillance, representing 14% of the under surveillance, and has since grown to be 10 sites and 1997). FoodNet began with 5 sites and 14.3 million persons under surveillance from those laboratories soon after they are diagnosed, and gather additional information, such as whether the person had traveled internationally before becoming ill, whether they were hospitalized for their illness, or whether they died.

Each spring, publication of the FoodNet report, with updated data on the incidence trends for nine pathogens and one syndrome provides an important index on the success of the national foodborne disease control programs, and on progress toward national health objectives. For example, in the most recently published summary from 2006, the incidence of diagnosed Campylobacter infections was 12.7 per 100,000, a significant decline of 30% from the baseline period of 1996—1998, and close to the national health objective for 2010 of 12.3 (CDC, 2007c). Similarly, in 2006 the incidence of E. coli O157:H7 infections in FoodNet was 1.3 per 100,000, a more modest, and statistically insignificant, decrease from the baseline period, and farther from the national health objective of 1.0 for that pathogen. For Salmonella, the incidence in 2006 was 14.8 per 100,000, hardly changed from the baseline period, and far from the national health objective of 6.8, indicating that much more effort is needed to even begin reducing these infections.

In addition to the annual feedback that this surveillance provides, we use this information to estimate the total burden of each of the acute infections. We know that many illnesses are not reported because the ill person did not seek medical attention, or because a diagnostic fecal specimen was not obtained, or because a laboratory did not examine the specimen for that particular organism. FoodNet conducts periodic telephone surveys of the population to determine the frequency of acute gastroenteritis, the frequency of care seeking behavior for milder or more severe infections, and through a survey of clinical laboratories the likelihood of specific tests being applied from the laboratory survey. From this information, we can reconstruct the larger number of illnesses that are represented by each diagnosed case. Thus for Campylobacter, we have estimated that each reported case represents 34 illnesses in all, the number by which the surveillance data can be multiplied to estimate the real incidence (Samuel et al., 2004). For Salmonella, we have estimated that the surveillance multiplier is 38 (Voetsch et al., 2004). Because identical surveys are conducted in the various reporting sites, it is also possible to see whether the shape of the reporting pyramid, and hence the surveillance multiplier, varies from site to site (Bender et al., 2004). The same approach can also be extended to compare surveillance data across different countries, offering a direct and simple means of adjusting for the marked differences in health care delivery, reimbursement, and reporting that can lead to large differences in the results of routine surveillance (Scallan et al., 2005; Vally, Kirk, Scallan, Hall, & Angulo, 2008).

While outbreak investigations can often determine the food source that caused the outbreak, it has been less clear to what degree these events reflect the sources of the much larger number of cases that are sporadic, i.e. that are not linked to a known outbreak. FoodNet provides a platform for conducting case—control and other epidemiological studies that can help determine risk factors and sources of sporadic infections. For example, FoodNet conducted a major
case—control study of sporadic Campylobacter infections, enrolling 1316 confirmed cases in 1998—1999 and matching them with 1316 healthy controls (Friedman et al., 2004). Each case and healthy control was interviewed with a standard questionnaire. The significant risk factors that were identified on multivariate analysis were used to determine the population attributable fraction of the cases: 28% were attributable to eating chicken or turkey in a restaurant, another 21% to eating other meats in a restaurant, 12% to foreign travel, 6% to contact with animal feces, 3% to drinking untreated surface water and 1.5% to drinking raw milk.

In a second case—control study that focused on the youngest age group, the exposures of 123 infants (<1 year old) with Campylobacter infection were compared to those of 928 healthy infants of the same age, as reported by their parents in telephone interviews (Fullerton et al., 2007). Not surprisingly, direct food exposures were not identified, though for infants <6 months old, breast feeding was strongly protective. An indirect exposure was important. Riding in a shopping cart next to fresh meat or poultry was a significant risk factor, suggesting that contamination of the outside of the packaging may be important. For infants between 6 and 11 months of age, visiting a farm or having a pet with diarrheal illness were risk factors for Campylobacter infection.

To study the sources of fluoroquinolone resistant (FQR) Campylobacter, FoodNet added resistance measurement to the large case—control study in some sites, so that a case—control study of FQR Campylobacter infection was possible. This study showed that these infections were also associated with eating chicken or turkey in restaurants, as well as with foreign travel (Kassenborg et al., 2004). A second FoodNet study demonstrated that those with the resistant infections had a longer duration of illness (Nelson et al., 2004). These observations contributed to the risk analysis that ultimately led to the withdrawal of approval of fluoroquinolones for use in poultry (Nelson, Chiller, Powers, & Angulo, 2007).

Other surveillance and research efforts are carried on through FoodNet. FoodNet is conducting case—control studies of sporadic infections with two rapidly increasing serotypes of Salmonella, Javiana and I 4,[5],12:i:-, which have become the fifth and sixth most common serotypes encountered in patients in the United States (CDC, 2007d). As part of a program to monitor antimicrobial resistance in enteric pathogens from humans, animals and foods, FoodNet staff systematically sample meat and poultry in retail stores for the frequency of contamination with Salmonella, Campylobacter and generic E. coli (see http://www.fda.gov/cvm/narms_pg.html). These samples have also been used to study the frequency in meat samples of vancomycin-resistant Enterococci, Clostridium difficile, and methicillin resistant Staphylococcus aureus. FoodNet also conducts operational evaluations of public health practice. A pathogen is identified in fewer than half of foodborne outbreak investigations in the United States. After this was shown to be most often related to a lack of adequate clinical samples, a simple specimen collection kit was deployed to rapidly collect and ship clinical samples to state laboratories in the event of an outbreak, greatly increasing the frequency of pathogen identification at modest cost (Jones et al., 2004). This can also contribute to the discovery of new pathogens. When no pathogen is detected even from the systematic study of good specimens, the samples are forwarded to CDC and to a FoodNet collaborating laboratory in Minnesota for an exhaustive search for emerging and new pathogens. The FoodNet population survey used to estimate the burden on gastrointestinal illness has also included questions about the consumption of different foods, which is helpful background information in the outbreak setting, to compare with exposure data collected from ill persons as part of hypothesis generation. If the ill persons are much more frequently exposed to one particular food than would be expected from the population background, more intensive questions focused on that food may reveal the source of the outbreak.

When entirely new foodborne disease challenges have arisen, FoodNet has provided a platform for rapid assessment that is critical to guide the development of longer-term surveillance strategies. Thus, in 1996, after cases of new variant Creutzfeld Jacob Disease (nvCJD) were reported from the United Kingdom, the FoodNet network mobilized to determine whether such case might be found in the United States as well. Within approximately two weeks, they contacted all the neurologists and neuropathologists in the surveillance areas, reviewed death certificates that reported Creutzfeld Jacob disease (CJD) between 1991 and 1995, and reviewed the associated pathology findings. They determined that there had been 94 cases of CJD in the catchment area. Only one patient was less than 45 years old, none were less than 30, and none were identified for which the pathology was consistent with nvCJD (CDC, 1996).

This information suggested that a centralized national surveillance would be the most effective approach to identify cases of nvCJD in the future, and this began later that same year. Arrangements were made to ship biopsy and autopsy materials of suspected CJD cases to the National Prion Disease Pathology Surveillance Center in Cleveland, Ohio, where they were evaluated and further characterized (Belay, Holman, & Schonberger, 2005). By 2002, more than half of all CJD cases occurring that year were being thus examined. By April 2005, prion disease had been confirmed in 1046 (60%) of 1747 patients tested, 85% of which were idiopathic CJD, 15% of which were familial CJD, and 0.5% were iatrogenic CJD. To date, only three persons with nvCJD have been detected in the United States, all three of which were born and raised outside the United States in countries known to have nvCJD.
The outbreaks we detect depend on the surveillance systems that we use

Detecting and investigating outbreaks of foodborne disease has been a central public health activity for many years. The usual outbreak scenario that appears in epidemiological training is a classic, highly focal outbreak, in which an egregious error in one kitchen leads to a high attack rate among the unfortunate victims. In such illustrative outbreaks, members of the affected group themselves are often the first to detect the outbreak; and investigation may reveal an important but local problem. Sophisticated surveillance is not required to detect these outbreaks, as they stand out dramatically from the background of sporadic cases. Of course, outbreaks really come in many shapes and sizes, and when they are less concentrated in space or time, or have lower attack rates, they are harder to detect. As our surveillance tools have improved, we have begun detecting much more dispersed outbreaks with growing frequency. The diffuse outbreak may affect many persons over a wide geographic area and across many jurisdictions. It may be the result of wide distribution of a food product that is uncommon, or that has a low level of contamination. In any one location, it may cause only a few cases, which are difficult or impossible to distinguish from the background noise of sporadic cases. These outbreaks are only detected if precise and standard subtyping systems are applied to the isolates of pathogens across many jurisdictions and if the data are rapidly collected and analyzed. This may reveal that one particular subtype has increased in several places. When such a cluster is detected, then epidemiological investigation can be targeted to those cases of the same subtype, to identify the exposures that cases may have in common. This requires coordination and standardization of the epidemiologic methods across multiple jurisdictions, which itself is a challenge. However, the benefit to public health can be substantial. Often these diffuse outbreaks are caused by systematic flaws in the food production process before the food is distributed — flaws that may be very difficult to detect by other means, and that may offer new and important insights into how to make the entire industry safer. One may place outbreaks on a spectrum — from the most concentrated and local events to the most dispersed and multi-jurisdictional, and consider that that many so-called “sporadic” cases may actually be part of small and highly dispersed outbreaks.

The value of subtype-based surveillance networks

As noted above, Salmonella serotyping was the first systematic subtyping system to be applied to any pathogen for routine public health surveillance, and it is no surprise that the history of food safety has been driven since then by innumerable informative Salmonella outbreaks, detected and investigated with the help of serotyping. Other subtyping systems have been used to provide further discrimination, such as phage typing or characterization of the pattern of antimicrobial resistance. However, in the last 10 years, a major new tool has been deployed: the pulsed-field gel electrophoresis (PFGE) pattern. This “DNA fingerprint” methodology is robust, can be applied to a broad variety of pathogens, and if rigidly standardized, can yield comparable results in many laboratories of a network. Since 1996, PFGE has been the foundation of PulseNet USA, the public health network for molecular subtyping of foodborne pathogens (www.cdc.gov/PulseNet). Each of the 50 State public health laboratories routinely perform PFGE on all clinical isolates of E. coli O157 and Listeria monocytogenes, and many isolates of Salmonella, as do the public health laboratories in a growing number of large cities, and the food laboratories in the regulatory agencies FDA and FSIS. These laboratories first pass a quality control certification, and then can upload the patterns that they determine to the national database, maintained at CDC. Participating laboratories can compare for themselves how their patterns match with others from their state, and those being uploaded from other states. CDC database managers also review the uploaded patterns for clusters of isolates that match, and thus may possibly be related. The number of uploaded patterns has increased to more than 45,000 per year in 2006, and the number of suspect clusters that were identified increased from 194 in 2004 to 302 in 2006.

Indeed it is a paradox to some casual observers, that with better attention to surveillance, the number of outbreaks detected will actually increase at first. The jurisdiction that reports few or no outbreaks is likely to have weak or absent surveillance, rather than remarkably safe food. In fact, each diffuse outbreak is likely to provide an opportunity to identify unsuspected problems, and to provide lessons that could improve food safety across an entire industry. That means that these surveillance and investigation networks are not optional research activities, but core capacities of any modern food safety system.

Such networks for subtype-based surveillance are being developed and adopted around the world, for an increasing number of pathogens. The Enter-net system, now coordinated out of the European Centre for Disease Prevention and Control (ECDC) in Stockholm provides coordinated surveillance based on Salmonella serotype across the member states, and has begun the deployment of the PFGE system as well. The PulseNet model has been deployed in Canada in the last decade as well, and a memorandum of understanding permits each country to consult the other’s database of PFGE patterns. PulseNet International, with regional grouping for Latin America, Asia/Pacific, the Middle East and Europe is now routinely exchanging patterns and information among a growing group of countries, paving the way toward a truly global network for subtype-based surveillance (Swaminathan et al., 2006). In parallel, the networks of national field epidemiology programs strive to develop uniform and collaborative investigative methods.
In the United States, the effort to develop more standard collaborative methods has led to an annual meeting of foodborne epidemiologists from all the states called, perhaps inevitably, “OutbreakNet”. In Australia, a combined program for active surveillance and collaborative investigation called OzFoodNet now includes all the states (OzFoodNet, 2006). In Europe, the better communication fostered by the transnational epidemiological training program EPIET now has a coordination focus in the ECDC, the logical locus for rapid coordination of investigations of disease problems that cross the national borders. Multinational outbreak investigations are now occurring regularly in North America, in Europe and in the Pacific region (Tauxe, O’Brien & Kirk, 2008). Recent examples include shigellosis transmitted by imported baby corn in Europe and Australia, salmonellosis from imported meats in Denmark and The Netherlands, salmonellosis from imported halvah and tahini sauce in Australia and several European countries, and Salmonella Enteritidis infections in Canada from almonds imported from the United States (Ethelberg et al., 2007; Isaacs et al., 2005; Kivi et al., 2007; Lewis et al., 2007; Unicomb et al., 2005). Indeed, though the smooth success of such investigations has not yet become routine, the outlines of what we can hope for are becoming clear, and the potential for multinational investigation of global outbreaks is at hand (Ammon & Tauxe, 2007).

**Outbreaks as the subject for surveillance**

Investigated outbreaks of illness are themselves quanta that can be reported and summarized as a surveillance activity. The first such summary of which I am aware, in 1920, reported 112 foodborne outbreaks in the United Kingdom, occurring between 1878 and 1918, most of which were caused by nontyphoidal Salmonella (Savage, 1920). In the United States, reports of investigations have been collected systematically though passively since 1967, and have been periodically summarized, and have served as the basis for a number of analyses (Cieslak et al., 1996; Olsen, MacKinnon, Goulding, Bean, & Slutsker, 2000; Sivapalasingam, Friedman, Cohen, & Tauxe, 2004). In recent years, this reporting has been made more active, leading to a doubling of the number of reports to 1200–1400 outbreaks per year, and has been converted to a web-based reporting, that permits swifter finalization and compilation (Lynch, Painter, Woodruff, & Braden, 2006) (www.cdc.gov/foodborneoutbreaks). As with case-based surveillance, the frequency and quality of reports may vary substantially across jurisdictions, depending on local resources, training, and competing priorities.

This new electronic Foodborne Outbreak Reporting System (eFORS) has also made possible more advanced information extraction, including the ability to rapidly summarize outbreaks from specific food commodities or outbreaks due to specific pathogens. We are now using it to estimate a general attribution of the burden of foodborne disease across the range of pathogens. We anticipate that updates of such calculations will be made routine, as they are now with the United Kingdom (Adak, Meakins, Yip, Lopman, & O’Brien, 2005). When subtype-based surveillance is applied to strains of a pathogen isolated from human, animal and food sources, it is also possible to construct a more detailed attribution of that pathogen, based on comparison of the distribution of subtypes (Hald, Lo Fo Wong, & Aarestrup, 2007). In the future, we anticipate being able to attribute Salmonella to animal reservoirs or environmental sources based on serotype and PFGE subtype distributions in isolates from humans and agriculture.

As we enter the 21st century, the challenge of foodborne diseases remains fresh and varied. We should expect new pathogens to be identified and to contaminate food in surprising fashions. As the market demands food that is fresh and varied year round, produced in more and more distant locations, and as the transnational trade in processed foods grows, the importance of multinational collaboration in detecting and responding to outbreaks becomes more important. If one country or region detects a problem that may be caused by a flawed production practice in another country, the public health of all counties is likely to benefit by a joint investigation and general dissemination of the results. Furthermore, the creation of a robust, multi-jurisdiction platform for active surveillance and for research can provide the information desired by the risk modelers, information that otherwise cannot be obtained during routine public health activities. Platforms like FoodNet or OzFoodNet require additional resources beyond what is routinely available in limited public health budgets, but can be supported by those who find the data most useful. Collecting and analyzing the reports of foodborne outbreaks can itself be useful for public health surveillance, and for guiding the direction of food safety. Robust and flexible surveillance methods, standardized and rapid pathogen subtyping networks, and collaborative epidemiological investigation strategies will be marks of a successful approach to foodborne infections in the coming years.

**Acknowledgements**

I am grateful for the contributions of many local state and federal health department officials for their efforts to maintain surveillance, and to investigate and prevent outbreaks. I thank Drs. Patricia Griffin and Peter Gerner-Smidt for their review of the manuscript. The findings and conclusions in this publication are those of the author and do not necessarily represent the views of the Centers for Disease Control and Prevention.

**References**


American Journal of Tropical Medicine and Hygiene, 77(5), 228.


Vally, H., Kirk, M., Scallan, E., Hall, G., & Angulo, F. (in press). Higher reported rate of Campylobacter infections in Australia than the United States: is this due to healthcare system differences? Emerging Infectious Diseases.

Challenges of quantitative microbial risk assessment at EU level

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Quantitative microbial risk assessment (QMRA) aims to model the fate of pathogenic micro-organisms along the food chain and the associated health risks. More importantly, it allows the \textit{a priori} estimation of the impact on public health of interventions in the food chain. The European Food Safety Authority is increasingly asked to provide scientific advice to the European Commission based on QMRA. Its application at the European level poses some unique challenges, both of a scientific and of an organizational nature. On the other hand, collaboration at the European level will lead to more effective use of limited expertise and resources.

Introduction

Quantitative microbial risk assessment (QMRA) is a relatively young branch on the risk analysis tree. Its development was stimulated by both political and technical factors. The agreement on the World Trade Organization (WTO—SPS) created a worldwide need for science-based standards for food safety. The recent “High Level International Food Safety Forum (Beijing, China; 26—27 November 2007) reaffirmed the need for transparent food safety regulations, based on risk analysis. Human dose-response models and predictive microbiology — two important components of any QMRA model — were proposed in the 1980s (Haas, 1983; Ross & McMeekin, 1994). The incorporation of variability and uncertainty — crucial in the modeling of any biological phenomenon — was made possible by the increasing availability of powerful (desktop) computers and dedicated simulation software in the 1990s. Based on these developments, principles and guidelines for food safety risk analysis were defined by the Codex Alimentarius Commission and specifically by the Codex Committee on Food Hygiene (CCFH) (Anonymous, 1995). Risk analysis consists of three separate, but interrelated stages: risk management, risk assessment and risk communication. CCFH is in the process of further developing and finalizing guidelines for these activities. In parallel, technical work on QMRA was started in several countries worldwide, in particular in the industrialized world. The World Health Organization (WHO) and the Food and Agricultural Organization of the United Nations (FAO) have coordinated technical developments worldwide by establishing the Joint Expert Meetings on Microbiological Risk Assessment (JEMRA). JEMRA has initiated a process to produce technical guidelines on the separate stages of the QMRA process (notably hazard characterization, exposure assessment and risk characterization) and has produced several international risk assessments, based mainly on a combination of modules developed for national risk assessments.

Within EFSA, the panel on Biological Hazards (BIOHAZ) deals with questions on biological hazards relating to food safety and food-borne diseases, including food-borne zoonoses, food hygiene, food microbiology, transmissible spongiform encephalopathies, and waste management. The final outcome of the work of the panel is usually reflected in an EFSA Opinion.\footnote{Information on JEMRA can be obtained from the websites http://www.who.int/foodsafety/micro/jemra/en/index.html and http://www.fao.org/ag/agfn/jemra_index_en.asp.}\footnote{For more information, see http://www.efsa.europa.eu/EFSA/efsano_locale-1178620753812_ScientificOpinionPublicationReport.htm.} Currently, BIOHAZ opinions in the
domains of food-borne zoonoses and food hygiene\(^3\) share many characteristics with risk profiles as defined by CCHF\(^4\): the risk profile is a description of a food safety problem and its context that presents in a concise form, the current state of knowledge related to a food safety issue, describes potential risk management options that have been identified to date, when any, and the food safety policy context that will influence further possible actions. The BIOHAZ opinions on food-borne zoonoses and food hygiene also provide qualitative assessments of risk. The step towards QMRA is in development and will be the focus of this review.

QMRA: what can it deliver, advantages and disadvantages

Even more than the estimation of the human health risk consequential to a pathogen–food product combination, the most important significance of QMRA is that it allows an \textit{a priori} assessment of the effect of intervention measures along the whole food chain, or combinations of intervention measures, on public health. Although a “farm-to-fork food chain model” is the often used terminology to describe the necessary type of model to achieve this, this neglects the important extension from exposure to human illness by effect (dose-response) modeling. In addition, when no interventions in the first part of the production chain (e.g. the farm) are considered, the model need not incorporate this part of the chain, and the model may, for example, become a “slaughter house to fork model”. An example of calculating the effect of intervention measures is the CARMA QMRA on \textit{Campylobacter} in chicken (Havelaar, Mangen et al., 2007), which showed that a combination of improvement of farm hygiene, reducing fecal leakage during processing and decontamination of carcasses with lactate is expected to yield a considerably lower human incidence of campylobacteriosis.

The value of QMRA for risk management is enhanced when it is combined with socio-economic analyses. This allows for selecting intervention measures that are realistically applicable in society, using criteria such as cost-effectiveness and public support (Mangen, Havelaar, Poppe, & de Wit, 2007). These analyses become extremely powerful when they are combined with disease burden methodologies introduced by WHO for the Global Burden of Disease project (Murray & Lopez, 1996). Disability Adjusted Life Years (DALYs) are used to integrate the effects of morbidity and mortality in one common metric, enabling the comparison of different diseases and outcomes. When combined with risk assessment and economic analysis, they allow the efficiency of intervention methods to be compared by assessment of the costs needed to avert a unit number (e.g. 1000) DALYs in the population (cost-utility analysis [Havelaar, Mangen, et al., 2007]). Such analyses can even provide a balanced comparison between interventions to control different pathogens.

A further advantage of the mathematical modeling in QMRA is that it also enhances our insight in the important processes in the food pathway, and highlighting knowledge gaps. Examples of this (again from CARMA) are the observations that lowering the concentration of a pathogen may be effective and more efficient than complete elimination, and that cross-contamination during slaughtering is basically a non-linear process. These findings for example imply that the estimated effects of interventions on human health risks should not be based on prevalence data alone. Mathematical modeling also helps to appreciate the complex dynamics of seemingly simple processes such as slaughtering.

In general, state-of-the-art QMRA does not allow for a precise estimate of human cases for pathogen–food product combinations, due to numerous sources of uncertainty along the food pathway and in the dose-response modeling. Typically these estimates are much higher then expected based on epidemiological estimates such as those from population-based cohort studies (e.g. Nauta, Evers, Takumi, & Havelaar, 2001), possibly because the dose-response relationship overestimates the probability of illness because acquired immunity is not accounted for (Nauta, Jacobs-Reitsma, & Havelaar, 2007). To address this problem requires interaction and integration of epidemiology and QMRA (Havelaar, Brüning et al., 2007).

An often heard criticism of QMRA is that it is extremely data hungry and/or time consuming. We do not agree with this criticism. The data need is not a consequence of the QMRA method, but of complexity of the questions that are asked and the degree of certainty that is required; in other words of the expectations that risk managers have of the QMRA method. A QMRA can in principle be simple when the question is simple or when the risk manager needs an order of magnitude estimate for initial risk ranking purposes. In such cases, point estimates can be used and simple tools have been proposed (e.g. Ross & Sumner, 2002). But the model cannot be simple when the question is, e.g., to estimate the effect on the number of human cases of, e.g., vaccination of production animals on the farm. This is a complex problem and it demands a full QMRA model. The large data need is then not a consequence of the method but of the complexity of the problem. QMRA is a good tool to structure the problem and helps identify the relevant data needs, but cannot be expected to give cheap and quick yet correct answers. Several recent questions presented to EFSA have highlighted the need to balance the expectations of the risk managers with the possibilities of QMRA, given current limitations of data availability, budget and time. Some examples will be discussed later.

\(^3\) For a discussion of risk assessments of transmissible spongiform encephalopathies, see the accompanying paper by Budka, Goossens, and Ru (2008).

With a QMRA model, proper sensitivity analysis can help to address the data problem by determining which parameters are important for the model output so that data collection can be focused on these parameters. Lessons on this important issue in QMRA may be learned from the related field of epidemiology. Here, much time and effort is put in large observational studies and the establishment and maintenance of complex databases. The food consumption surveys to support nutritional epidemiology are a case in point. Efforts are emerging to include the needs of chemical and microbial risk assessment in such studies. There is not a lack of data collection in the EU, as the EFSA reports on zoonotic agents illustrate. The challenge is to achieve effective communication between those responsible for monitoring and surveillance programs and those responsible for risk assessment.

In practice, quantifying the uncertainty of the model output of QMRA is complex or even impossible. Partly, this is a theoretical and model implementation problem: estimating uncertainty becomes complicated when the QMRA model is complex. For example, in food chain models it may be necessary to include different types of variability, e.g. within and between batches of the food product. Uncertainty then needs to be superimposed on this already complex model, resulting in infeasible or computationally extremely demanding models. Uncertainty is also a data problem. The uncertainty of parameter values that are fully unknown cannot be assessed and the same may hold for estimates based on expert opinion or estimates based on microbiological data from laboratory experiments? A similar problem is the quantification of the uncertainty of the dose-response relationship based on data from only one or a few strains and tested on a selected group of (healthy) volunteers. Realizing this, however, is not a drawback of QMRA but an advantage: it clarifies the difficulties associated with limited knowledge that may be ignored otherwise and, as a consequence, lead to incorrect management decisions. Solving the uncertainty problem is one of the main challenges of QMRA in the near future.

**EFSA and QMRA**

EFSA is developing a strategy on QMRA taking into account expectations from the European Commission (EC), Member States (MS) and scientists, the expected advantages and disadvantages, the available resources at European and national levels and the international experience. A consultation among all interested parties was held in 2004—2005. It was concluded that there was broad support in the EC, among MS and scientists for development of QMRA at the European level by EFSA. Some expected advantages were general for QMRA. These included providing a better basis for objective, risk-based criteria and targets, to promote risk-based decision making, to improve risk communication, and to focus data collection efforts.

Other expected advantages were specific for its application at the European level. These included facilitating the free movement of goods, the optimal use of scarce resources, strengthening the position of the EC in Codex and the World Trade Organization and knowledge transfer. Expected disadvantages of a general nature were the time and resource intensive nature of QMRA (but see the discussion earlier in this paper), current models not being sufficiently realistic to reflect the complicated technological and biological processes, and the risk of wasting resources if questions were not sufficiently focused (indicating the need for effective interaction between risk managers and risk assessors). Specific disadvantages at the European level included the difficulty of adequately reflecting regional differences in food production and consumption, a lack of harmonization of models, many MS not yet being able to contribute and the possibility that too much emphasis would be placed on differences between MS.

Based on the results of the consultation, EFSA and the EC have discussed potential applications of QMRA to support European food safety policy, and several mandates have been received since 2006. Some questions dealt with by EFSA will be discussed in this review, together with some other examples from the authors’ work that are relevant for decision making at the European level.

**Examples of QMRA at the European level**

Salmonella in meat

This opinion of the BIOHAZ panel (Anonymous, 2008a) was based on terms of reference from the EC to EFSA to assess:

“The relative contribution of different meat categories, such as carcasses, fresh meat and products thereof, minced meat and meat preparations to cases of foodborne Salmonella infections in humans, taking into account the occurrence of the pathogen in the food chain, risk factors, food production flows and food preparation and consumption habits. A distinction between meats derived from different species, such as bovine, porcine, poultry (if possible separately broilers and turkeys) and other possible species should be considered. In particular, the impact of the intended and common use of the abovementioned meat categories derived from different species should be taken into account as well as the impact of cross-contamination.”

Basically, this is a risk attribution question. Attribution can be performed according to two approaches, bottom-up or top-down. In the bottom-up approach, multiple risk assessments are carried out for one or more agents in one or more food products as the basis for comparing the consumer risks. This can be done with relatively simple QMRA models as these need not evaluate the public health effect of measures, which allows for comparing many food products,
and even transmission via direct contact with animals and via water can be included. The technique of comparative risk assessment gives on the one hand more insight in the relative effect on public health of interventions in one specific transmission route, and on the other hand it can serve as a first step of a general QMRA for a pathogen, as it can pinpoint the most important transmission routes to focus on with an extensive full scale QMRA model.

A well-known example is the US FDA/FSIS *Listeria monocytogenes* in different ready to eat foods. This study identified deli meats as the most risky products in relation to food-borne listeriosis in the USA. It is interesting to note that high population risks can be related to high risks per serving (e.g. paté and meat spreads) but also to high consumption of foods with relatively low risks per serving (e.g. pasteurized milk). An example that included comparison of different food routes with non-food routes for *Campylobacter* spp. was published by Evers, Van Der Fels-Klerx, Nauta, Schijven, and Havelaar (2008). The results suggest that raw food consumption and direct contact with animals are significant transmission routes. The bottom-up approach has not yet been applied to human salmonellosis.

In the top-down approach, the incidence and outcomes of enteric illness are taken from epidemiological surveillance. In Europe, data on the incidence of infectious disease are collected by the Basic Surveillance Network, coordinated by the European Centre for Disease Control and Prevention (ECDC). EFSA and ECDC report data on zoonotic antimicrobial Resistance and Food-Borne Outbreaks in the Community Summary Report on Zoonoses, Zoonotic Agents and Antimicrobial Resistance and Food-Borne Outbreaks in the European Union. Data in this report are mainly based on passive, laboratory-based surveillance and represent only a small proportion of the total illness occurring in the population. This proportion varies between countries, depending on the health care system and other factors. Relatively few attempts have been made to calibrate these surveillance systems. This lack of information is one of the main problems in obtaining a better insight in the burden of infectious disease in Europe.

Once incidence data are available, the next task is to attribute the observed illness to different (food) sources. With regard to attribution of salmonellosis, some studies have been performed by individual MS, using different data sources and methods. These included outbreak investigations, analytical epidemiology (in particular case-control studies), microbial subtyping and elicitation of expert opinion (Anonymous, 2008b). No studies have been performed at the European level, and data for such studies are incomplete. Even where data are available, they are not always fully analyzed in particular to identify the main sources of human salmonellosis (source attribution). Most cases of human salmonellosis are considered to be foodborne. It was only possible to provide a qualitative ranking of different food groups. In the EU, eggs and egg products are still the most frequently implicated source of human salmonellosis. Meat is also an important source of food-borne salmonellosis, with poultry and pork being implicated more often than beef and lamb. More specific conclusions about the relative importance of specific meat categories as requested by the EC, for example fresh meat, minced meat and products thereof, cannot be made at the present. It was concluded that for this question, the development of a bottom-up QMRA approach was the most feasible option. To support such an approach, representative data on the prevalence and (variability in) concentrations of *Salmonella* in retail products must be available for all products to be compared in specific scenarios agreed upon by both risk assessors and risk managers. In addition, data on food storage, handling, preparation and consumption that reflect the diversity of consumer habits in the EU should be available. The EU baseline studies on the prevalence of zoonotic pathogens in food animals are an important step in this direction and should be extended to cover other parts of the food chain.

Salmonella in pigs

In EC regulation no. 2160/2003, provisions are laid down for Salmonella and other food-borne agents that pose a public health risk. The Regulation aims at setting targets for the reduction of the prevalence of zoonotic agents at different stages of the food production chain in member states. Before setting a target for Salmonella in pigs, a cost-benefit analysis must be performed. According to the Regulation, EFSA must be consulted prior to setting a reduction target. Therefore, the BIOHAZ Panel was requested to carry out a quantitative microbial risk assessment (QMRA) of Salmonella in pigs, as this is a necessary condition in order to be able to perform a cost/benefit analysis. Baseline studies on Salmonella in pigs are executed at present, providing essential data for QMRA modeling.

In the framework of article 36 of its founding regulation, EFSA launched a call for proposals entitled ‘Quantitative microbial risk assessment on Salmonella in slaughter and breeder pigs’. The awarded consortium (VLA, RIVM, FOOD-DTU) will produce a QMRA, which will be used by the BIOHAZ Panel to formulate their opinion. This is the first time that the ‘article 36 procedure’ is applied, using the list of expert organizations drawn up in December 2006 on the basis of national nominations. Being the first time, a number of teething troubles can be identified:

- it took a long time before the call for proposal was published on the EFSA website (from October 2006 to April 2007). In combination with a lengthy decision

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and contracting procedure, this implied that the start of this 18-month project had to be postponed from October 2007 to January 2008. Hence, preparations for the contract took almost as much time as the anticipated duration of the QMRA (which is typically criticized for being time consuming);
- due to the fact that EFSA only partially funds direct costs and, more importantly, only pays a low percentage of indirect costs, substantial national co-funding had to be obtained, for which no mechanism was yet in place;
- QMRA scientific expertise is a very limited resource in Europe, in terms of persons and institutions. This easily leads to potential conflicts of interests between Article 36 consortia and EFSA panels and working groups, hampering the utilization of experts.

The QMRA for Salmonella in pigs will encompass a probabilistic farm to fork (and beyond) food chain model for the entire EU, where variability will and uncertainty will not be quantified. The considered outputs will be prevalence and/or numbers of Salmonella on pork meat and the number of human cases of salmonellosis. The model will be divided into a number of modules that are allocated to the different institutions. The modules will be (1) farm, transport and lairage, (2) slaughter and processing, (3) preparation and consumption, and (4) hazard and risk characterization. The following interventions included in EFSA’s Terms of Reference will be considered:

- the expected reduction of Salmonella cases in humans (or pig meat at retail) by a reduction (e.g. 5- or 10-fold) of Salmonella prevalence in slaughter pigs, based on bacteriology in lymph nodes or serology at slaughter. It must be noted here that for QMRA it is not these measurements that are essential, but Salmonella prevalence and numbers in feces and on hides. The QMRA will attempt to use a dynamic model of on-farm infection (Hill, Snary, Arnold, Alban, & Cook, 2008) to combine the different sources of information on Salmonella infection of slaughter pigs;
- the expected reduction of Salmonella cases in humans (or pig meat at retail) by a reduction of Salmonella prevalence in piglets from breeder farms;
- the reduction of the prevalence in slaughter pigs by the most important potential treatments or control measures at farm level (including sources of infection of fattening pigs);
- the expected reduction of Salmonella cases in humans (or pig meat) by the most important control measures during transport, at lairage or during the slaughter process.

Many national QMRAs have been completed or are underway. The information and expertise obtained in these QMRAs must be utilized in terms of QMRA models, available data, and potentially useful interventions. This will be achieved by building a network of scientists, placing a call for data on the EFSA website and organizing workshops on QMRA and data.

A novel feature of this QMRA is that the whole of the EU will be considered, including differences between member states. These differences are expected to be large, and related to pig production systems (conventional, large-scale), as well as slaughtering and processing methods (large industrial scale, traditional methods) and eating habits (kinds of products and ways of preparation). As much as resources allow, this variability will be built into the model. The way to do this is still under discussion, but grouping of countries and selecting indicator food products are being considered. In this discussion, data availability also plays a role: this is expected to show large variation between EU countries.

Import and export of pigs and pork also has to be considered in this QMRA. Intra-community trade between EU member states may be less important at the EU level than at the national level. Import from outside the EU does matter: interventions undertaken on EU farms will not influence the safety of imported pork and therewith the risk to EU citizens. Export outside of the EU matters also: interventions undertaken in the EU will have less effect on EU citizens when there is more export, but may impact the competitive position of the European pork industry that needs to be considered in the subsequent cost-benefit analysis.

Campylobacter in broilers (model harmonization)

Thermotolerant Campylobacter spp. are a leading cause of zoonotic enteric infections in most developed and developing nations worldwide. In the European Union (EU), Campylobacter was the most commonly reported gastrointestinal bacterial pathogen in humans in 2004 and 2005. In the past decade, several national quantitative risk assessments for Campylobacter in broiler meat have been developed to support risk managers in controlling this pathogen. A first international activity where several available risk assessments were combined was organized by FAO and WHO (Anonymous, 2003). Recently, within the European Network of Excellence Med-Vet-Net, a group of scientists has explored the feasibility of the construction of an European consensus model of Campylobacter in broiler meat, based on the existing models. Ideally, such a consensus model would provide a tool for individual member states to perform their own risk assessment. Also, it would allow for a harmonized approach of European risk management of the problem.

Models developed in the United Kingdom (Hartnett, Kelly, Newell, Wooldridge, & Gettinby, 2001), Denmark (Rosenquist, Nielsen, Sommer, Norrung, & Christensen, 2007), France (Kashket, Smadja, Bakshi, & Naval, 2005), and other countries have been considered for this consensus model. This resulted in a harmonization process, with the aim of building a model with a common structure and model parameters that allow for a comparison of risk assessments at the national level. Interactions between pathogens within the gastrointestinal tract, the effect of interventions at different stages of the production chain, and the impact of changes in the feeding habits on the risk of Campylobacter infections in broilers were included in the model. The model was validated against data from different countries, and the results were compared with the results of national risk assessments.

References

2003), the Netherlands (Nauta et al., 2007) and Germany (Brynestad, Lubet, Braute, & Bartelt, 2008) have been compared to identify their differences and similarities (Nauta et al., submitted for publication). It was found that, although the dynamics of the existing models may differ substantially, some conclusions are shared by all. An important finding is that all risk assessments conclude that the most effective intervention measures aim at reducing the Campylobacter concentration, rather than reducing the prevalence. This insight is a typical result from QMRA activities and broadens the potential for (more cost-effective) interventions.

The group concluded that it is neither feasible nor desirable to merge the different models into one generic risk assessment model. This is largely a consequence of the fact that the most important attribute of a microbial risk assessment is that it is “fit-for-purpose” (Lammerding, 2007). The purpose of risk assessments may vary between individual countries and that of a generic model has yet to be defined at a European level. Also, the variety in farm systems, industrial processing and most importantly the large variety in practices related to consumer food preparation and consumption, complicates a unified approach.

As an alternative to the construction of a consensus model, the scientists in Med-Vet-Net are now developing a framework that, based on the existing models, may offer guidance to those who wish to develop their own risk assessment model. Ideally, this framework is maintained at the European level after its development in the Med-Vet-Net project. Relevant areas for further research to improve the quality and practicality of QMRA are the integration of data- and mechanistic-based broiler processing models, and definition of minimum requirements for models describing the pathogen transfer and survival in the consumer phase of the farm to fork chain.

European risk management of the Campylobacter problem could benefit from an improved interaction between the establishment of surveillance and monitoring programs and quantitative risk assessment activities. Here, a clear statement of purpose by risk managers should be the starting point of QMRA. The data needs are then identified by QMRA, and those, once checked for practicality, will define the most effective points in the food chain for monitoring and surveillance.

Risk-based standards

New approaches to food safety management are being developed by CCFH, taking into account the Sanitary and Phytosanitary agreement under the World Trade Organization. The Appropriate Level of Protection (ALOP) plays a pivotal role in the SPS agreement. It is the current level of consumer health protection, as offered by existing food safety systems. It is not some future goal (Anonymous, 2002). To implement the ALOP concept in food business operations, several new concepts were deemed necessary. These include the Food Safety Objective (FSO, the prevalence and/or concentration of the hazard at the moment of consumption), the Performance Objective (PO, the hazard level at an appropriate point in the food chain) and the Performance Criterion (PC, the effect of a process on a food-borne hazard). These new concepts have been defined alongside existing microbiological criteria, which still play an important role in the implementation of food safety systems in practice.

Whereas traditional microbiological criteria were based on the level of hazard control that was feasible in well-operated food production systems, the new metrics (and microbiological criteria derived from them) should explicitly be linked to consumer risks. QMRA is the appropriate tool to provide this link between hazard control and consumer risk.

Different approaches have been proposed to apply QMRA in the setting of food safety criteria, and this field is still actively developing. One example is presented by Nauta and Havelaar (2008), based on the model for Campylobacter in the broiler meat chain, developed in the CARMA project. The model suggests that reducing the number of Campylobacter on broiler meat is an efficient way of reducing human campylobacteriosis. There are different ways of achieving this goal, including improved slaughter hygiene, decontamination of carcasses and scheduling. The latter approach is based on the observation that in live broilers, the variation in the level of Campylobacter between different flocks is higher than the variation within flocks. Thus, if highly contaminated flocks were detected and diverted from the fresh meat chain (scheduling), a reduction in the level of contamination of meat and of consumer risk is to be expected. The risk assessment model was used to estimate the effects of testing at different stages of the food chain (at the farm, at the entrance of the slaughter house or after cooling) and using different test sensitivities. Basically, the results present the risk manager with a tool for balancing the risk reduction for consumers against the costs for industry (i.e. the percentage of scheduled flocks).

Challenges and opportunities for QMRA at the European level

QMRA has the potential to develop into a practical and important decision support tool for Community food safety policy and EFSA should play a key role in further developing and coordinating QMRA at the European level. To reach this goal, several challenges exist.

Organizational challenges

Organizational challenges include the need to improve the dialogue with risk managers (primarily at the European Commission) to develop pro-active, focused questions. Current questions are usually very broad and may lead to complex and resource demanding models. To fully profit
from the power of QMRA, and in particular its iterative nature, questions should be formulated well before the policy needs to develop regulations arise. This will also make it possible that model development actually takes place before data collection, resulting in more focused surveillance and monitoring activities. It must be borne in mind that developing a mathematical model is cheap in comparison to collecting data, certainly across the whole of the EU. The potential of QMRA to focus data collection efforts is currently not used because data collection usually precedes model development instead of the reverse, which would be far more efficient.

There is limited capacity and expertise to develop and apply QMRA in Europe. Even though the capacity is growing, there is an urgent need to foster international collaboration. Learning from developments in other countries by participation in joint projects at the European level is far more preferable than duplicating the development of models in different member states. It will also lead to harmonization of QMRA in a more natural way.

QMRA results demonstrate more explicitly than before that zero-risk is not attainable. This implies that risk managers are increasingly faced with the need to develop more explicit approaches to defining tolerable risk, in relation to economic and social pressures and to translate this into tolerable hazard levels (prevalence and concentration) at different points in the food chain.

Scientific challenges

A key need in any risk assessment is the availability of appropriate data. Such data should be representative of the system under study, and adequately reflect the variability of microbial contamination in these systems. Structured, harmonized data collection is a prerequisite and significant progress has been made in recent years. The baseline studies on the prevalence of zoonotic pathogens in primary production for the first time provide comparable data across the EU. In addition to the prevalence, data should be available on concentrations of pathogens at representative steps of the food chain. Such data are less variable between countries and in time, and therefore study designs which are less ambitious than the baseline studies can be applied. Their design should be based on identified needs in risk assessment and be developed in close collaboration between risk assessors, laboratory scientists and risk managers. As discussed before, a pro-active approach is necessary as tomorrow’s risk assessments should define today’s data collection efforts.

Quantitative information on pathogen behavior (pathogen characteristics in combination with environmental characteristics) in the food chain is a crucial input in risk assessment models. Here, even more than in other fields, the models should guide data collection. Food production systems are highly complex and adequate study designs need to take this complexity into account. Descriptive analyses, where samples are taken and results compared without predefined hypotheses about the system dynamics usually do not yield sufficient information.

Detailed information on food consumption and preparation practices is necessary to adequately model the consumer phase. Current food consumption surveys do provide very useful information on meal composition, which can directly be used for QMRA. However, the dynamic nature of microbial contamination in the kitchen (growth under suboptimal storage conditions, die-off due to cooking and cross-contamination) create a need for more detailed information on consumer handling practices. Partly, this information can be obtained by extending the questionnaires of food consumption surveys; partly there is a need for specific, observational studies.

To improve the timeliness of scientific advice based on QMRA, pro-active model development is necessary. By using a modular approach, basic elements of food chain models should be developed in such a way that they can effectively be combined for a complete and case-specific food chain (Nauta, 2007). It is essential to explore when simple (e.g. deterministic) models suffice and when more complex (stochastic) models are necessary. The debate on this issue is currently most active in relation to the new metrics defined by CCFH (FSO, PO, PC, see elsewhere).

Variability between MS is a challenge for any risk assessment, and also for QMRA. It will not be possible to include all existing food production, processing, preparation and consumption practices across the EU in a risk assessment. A series of “typical” products and processes should be defined. As this is partly a subjective choice, which may influence risk management decisions, these defaults should be agreed between risk assessors and risk managers.

The role of EFSA in QMRA at the European level

Important tasks for EFSA to stimulate the development of QMRA at the European level were identified in the 2004–2005 consultation. A first task is to create a network of European institutes for QMRA, to foster international collaboration and to promote exchange of data and models. In this network, harmonization of QMRA models should be a principal target. Whereas any risk assessment should be “fit-for-purpose” and thus be built to answer a specific question, the efficiency of the process can be greatly improved by utilization of agreed modular approaches. The article 36 procedure is not very flexible for this purpose and alternative solutions may be considered. Seeking cooperation with existing networks (e.g. Med-Vet-Net and JEMRA) may be a first step in this direction. EFSA may also seek to promote the support of QMRA networks through other EU funds, e.g. through DG Research or DG Sanco.

A second task for EFSA is to develop and maintain databases to support QMRA. Such databases are now established for the production of the annual zoonoses reports. Whereas these reports provide a useful overview of the currently available data in the EU, their usefulness is limited as they were collected by different methods and according to
different sampling schemes. Improving the data quality in the zoonoses reports is a stated priority for EFSA. Recently, the BIOHAZ panel presented opinions on the monitoring of Toxoplasma, Yersinia and Vero-toxin producing Escherichia coli. The recommendations in these opinions will be the basis for discussions with member states about monitoring of these pathogens and reporting of results. Likewise, EFSA is evaluating the existing food consumption surveys from the needs of risk assessment, which will lead to recommendations on possible amendments and additions.

References
BSE and TSEs: Past, present and future

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Bovine spongiform encephalopathy (BSE) in the UK and variant Creutzfeldt-Jakob disease as result of BSE transmission to humans caused worldwide concern that increased when active surveillance proved BSE to be widespread in continental Europe. Science-based control measures by the EU restricted the BSE epidemic to a continuous decline over recent years. However, the future has to consider the global BSE situation, the feasibility of animal TSE eradication, the zoonotic potential of scrapie and CWD, the risk from atypical strains, and secondary transmissions between humans e.g. by blood. It is time to be re-assured but yet too early for complacency.

“Let us hope that it is not true; and if it is true, let us hope that it does not become widely known”

— Lady Ashley —

on Darwin’s theory of mankind’s descent from ape-like creatures.

Introduction

Like with Darwin’s theory, the truth has been more than unwelcome in the case of transmissible spongiform encephalopathies (TSEs) or prion diseases. They comprise cause untreatable, deadly diseases in humans and animals and have taken, with bovine spongiform encephalopathy (BSE, mad cow disease) as a man-made disaster, a tremendous toll in political and consumer trust leading to economic costs in addition to individual suffering. TSEs in humans include Creutzfeldt-Jakob Disease (CJD) and Kuru. TSEs in animals include BSE in cattle and scrapie in sheep and goats. Scientifically, TSEs are unique in biomedicine: they occur by spontaneous neurodegeneration, genetic aberration, or infection with the TSE agent (prion). The particular scientific challenge is why TSEs are infectious. Intensive research in the past 20 years identified the pivotal role of a normal cellular protein, the prion protein (PrP) that accumulates in TSE brains in an abnormal isoform called PrPsc (sc from scrapie as prototype of a well studied TSE). More recent evidence has suggested that PrPsc is the major if not only component of the prion; however, several issues still need to be clarified, e.g. a major discordance between the levels of PrPsc and infectivity (Silveira et al., 2005).

The past

The history of TSEs comprises distinct periods, from ignorance and later understanding leading to mobilisation of resources for scientific evidence and protection, to re-assurance and current indications of complacency. Since the first description of scrapie in the 18th century until the 1980s, TSEs remained hidden within an obscure niche of human and animal neuropathology. The emergence of the BSE epidemic in the UK since the middle of the 1980s has put TSEs out of the shadow. To date more than 190,000 cattle have been diagnosed with BSE, mainly in Europe but also some non-European countries. Inclusion of mammalian Meat and Bone Meal (MBM) contaminated with prions into cattle feed is widely considered to have caused the BSE epidemic starting in the UK (Wilesmith, Ryan, & Atkinson, 1991). In 1996, monitoring of human TSEs revealed the occurrence of a new type, a variant form of Creutzfeldt-Jakob disease (vCJD) (Will et al., 1996). The most likely source for human infection was considered to be the consumption of bovine meat products contaminated with BSE, thus identifying BSE as a zoonosis. The considerable political and social impact of this and the initially associated predictions of a large human epidemic were further exacerbated in 2000 when active
surveillance of BSE proved that its occurrence was much more widespread in continental Europe than was admitted at government level.

Mobilisation of huge financial resources by the EU level leading to an increased scientific output resulted in science-based control measures by the EU and elsewhere. These measures were the base for the development of key legislation to protect human and animal health from the risk of BSE. EC Regulation 999/2001 lays down rules for prevention, control and eradication of certain TSEs and has since been continuously reviewed in the light of new scientific evidence, the evolution of the TSE prevalence and the practical implementation in the field.

The present
Present animal and public health issues comprise the development of BSE over time in the world, whether control measures taken are sufficient and may lead to eradication of the disease, and the development of vCJD over time in the world, in particular with regard to secondary transmissions by blood.

BSE in the world
The European Food Safety Authority (EFSA), through its Geographical BSE Risk (GBR) assessments (European Food Safety Authority, 2006), has played a major role in assessing, predicting and ultimately proving the spread of BSE in EU member states (MS) and third countries. The GBR is an indicator of the BSE risk in a given country or region. The Scientific Steering Committee (SSC) of the European Commission (EC) developed this GBR methodology (SSC GBR) in 1998. As a result of its application, firstly by the SSC itself and from 2003 by EFSA, all EU Member states and a number of non-EU countries have been classified within four qualitative categories.

As MBM, the key carrier of BSE infectivity, has been exported from EU countries to third countries all over the world with a maximum of exports during the peak of the BSE epidemic (Lord Phillips (chair), 2000), and re-exported from these third countries, there is a global BSE scenario that is impossible to verify, as many developing countries have no BSE surveillance system in place. However, even in the absence of a surveillance system, the GBR assessments have clearly indicated for most countries assessed that recycling and amplification of the BSE agent in their domestic cattle population is taking place at different levels, highlighting a dormant but explosive problem. This GBR (European Food Safety Authority, 2006) has been assessed for 65 countries, and models two main components: (a) the external challenge, which is defined as both the likelihood and the amount of the BSE agent entering into a defined geographical area in a given time period through potentially infected cattle and/or MBM and (b) the stability of the internal system, which is defined as the ability of the internal BSE/cattle system to prevent the introduction and, if introduced, to eliminate or reduce amplification and spread of the BSE agent within its borders. The GBR assessment has proven to be very accurate and spectacularly succeeded in predicting presence of BSE in several countries including EU countries before it was first reported or even before the surveillance system was put in place.

In the following section that is illustrated with supportive graphs, we try to understand distribution, trend and future evolution of the worldwide BSE epidemic by surveillance data to calculate prevalence and incidence estimates. Surveillance is divided into (1) active, i.e. post-mortem testing of cattle that are not notified as BSE suspects but belong to two categories, i.e. healthy slaughtered vs. risk animals (fallen stock, ill animals or emergency slaughtered); and (2) passive, i.e. mandatory reporting and testing of BSE suspects (cattle that are notified because of clinical signs compatible with BSE). The prevalence is obtained as the number of cases identified through active surveillance per 10,000 animals tested. Incidence rate estimates are calculated as the number of new cases diagnosed by passive and active surveillance per million live animals (i.e. cattle aged over 24 months) per year or for otherwise specified time periods. The estimates of descriptive epidemiology are determined by comparing the BSE distribution by geographical location and time. The focus is on the period since 2001 when massive active surveillance was enforced within the EU, and on six main areas where BSE cases were reported, namely the United Kingdom (UK), the 14 earliest EU Member states (new EU-MS, i.e. the 10 countries that joined EU in 2004), Switzerland that first devised active surveillance, Canada and US together, and Japan. To calculate annual incidence rates throughout the world, data on cattle populations and the number of BSE cases (where found) for each country were obtained from the EU (European Commission Health & Consumer Protection Directorate-General, 2007) or from a number of official web sites (mainly of the National Agricultural Authorities). Incidence data (crude rates) were available for all areas, whereas prevalence data (age & risk category specific rates) only for EU areas. Relative risk estimates were obtained through calculation of incidence rate ratios (IRR) and their 95% confidence intervals (CI) fitting a Poisson regression model and using the group of the new EU-MS as reference category. When comparing IRRs by geographical areas and year, the incidence of 2007 of the group of the EU14 was used as reference category.

Between 2001 and 2007, a total of 7734 BSE cases were registered of which most cases occurred in the EU (7588) with and in particular 50% of these in UK. In the rest of the world, the number is really small (Switzerland 98, Japan 34, Canada 11, USA 2, and Israel 1) but, as it happened and was proven in the EU, even few cases may have a tremendous effect. With regard to the spatial distribution of the risk over the period of 2001–2007, taking the 10 new EU-MS as reference category, there is a similar incidence
rate ratio for Japan, but there is still a 6-fold higher risk for Switzerland and the EU14, and a 40 times higher risk for the UK, whereas the risk for the North American countries is very small (Fig. 1). When looking at the trends of the incidence, we can detect two different groups: UK, EU14 and Switzerland show decreasing trends, whereas, even if based on small figures, the other three areas are still showing an increasing trend up to 2006 (Fig. 2). The temporal evolution of the risk over the period of 2001–2007 (assuming a risk of 1 for the EU14 in 2007) is shown in Fig. 3. In term of relative risk, the time trends show: a low, although increasing risk for the American countries, whereas in the rest of the areas the level of risk is converging to that of EU14 in 2007. Comparing the absolute number of cases with the year of birth that is the likely year of infection (Fig. 4), Switzerland, UK and the EU14 countries show a clear peak around the mid nineties, whereas there is a shift towards later years of birth when looking at Japan, North America and the new EU-MS. These countries share a couple of major peaks, with a gap of 5 years corresponding to one incubation period, indicating a second wave of infections around the year 2000. The two different kinds of trend are confirmed when analysing the prevalence by birth cohorts (Fig. 5). In the EU14 the prevalence, cohort by cohort, is progressively declining, but what we see in the new EU-MS, after a peak in 1996–1997, is a decrease in the 1998 cohort and a second wave starting with the 1999 birth cohort.

**BSE controls and eradication**

The main protective measures in EC Regulation 999/2001 include: (a) a ban on inclusion of MBM in feed for animals farmed for food production; (b) a comprehensive surveillance system including *post-mortem* testing and (c) compulsory removal and destruction of Specified Risk Materials (SRM) containing the highest risk of BSE from animal carcasses. More than 4.5 million cattle were slaughtered and destroyed to prevent further spread of the disease. Indeed, the measures succeeded in restricting the BSE epidemic to a continuous decline, resulting in re-assurance and even, more recently, complacency (Anonymous, 2003). Over the past 6 years, more than 61 million adult bovine animals have been tested in the EU using one of the EU approved BSE tests, with around 7500 cases detected. A constant decline of about 30% per year in the number

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**Fig. 1.** Comparison of BSE incidence rate ratios in different regions/countries 2001–2007, with the EU new MS as reference category, log scale. “EU14” are the 15 earliest MS without UK; “new MS” are those entering the EU in 2004.

**Fig. 2.** Temporal evolution of BSE incidence in different regions/countries since 2001 in cases per million bovines aged over 24 months. “EU14” are the 15 earliest MS without UK; “new MS” are those entering the EU in 2004.
of cases has been recorded. This is combined with mounting scientific evidence on the sources of the epidemic, which seems to be put under control by the measures put in place as observed by the continuous decline of positive cases. Thus, it is not surprising that economic and political pressure has emerged for relief from costly precaution measures. As guidance, the TSE Roadmap (European Commission, 2005), developed by DG SANCO and adopted in 2005, provides an outline of possible changes to these measures in the short, medium and long-term future.

While control measures usually aim to eradicate an infectious animal disease, it is getting less and less probable that this can be achieved with TSEs: active surveillance has identified atypical cases that might well be spontaneous in origin, similar to sporadic CJD in humans. Atypical BSE now comprises an L- and H-type, named according to the abundance of high or lower molecular PrPsc bands at immunoblotting, and is more difficult to diagnose by conventional rapid test during slaughter than classical BSE (Baron, Biacabe, Arsac, Benestad, & Groschup, 2007).

vCJD in the world

As of June 2008, 208 vCJD cases have been reported, including 167 in the UK, 23 in France, 4 in Ireland, 3 each in the USA and Spain, 2 each in The Netherlands and Portugal, and 1 case each in Italy, Saudi Arabia, Canada and Japan (EUROCJD, 2008). While the majority of cases seem to have originated from a local BSE source, all 3 cases of the USA, 2 Irish cases, and 1 case each in France, Canada and Japan have been attributed to acquisition outside of the country, mostly in the UK. In the UK, the vCJD epidemic reached a peak in the year 2000 and has since declined to a current incidence of about two diagnoses/deaths per year. There have been modelling approaches to predict the future trend; extrapolating the best fitting model (the quadratic model) gives an estimate of 1 death in the next 12 months (95% prediction interval 0—4) (Andrews, 2008). Four cases of transfusion associated vCJD infection have been reported to date, who received blood from earlier cases, indicating that transmission by blood is a very effective route for vCJD prions. This is bad message for the UK, because a study of archival lymphoid tissue samples in the UK suggested a prevalence of about 4000 persons aged 10—30 years who are PrPsc positive (Hilton et al., 2004). At variance with clinically manifest vCJD cases who uniformly have had a homozygous M/M genotype at codon 129 of the PrP gene that is a susceptibility factor for all types of human TSEs, also V/V and M/V genotypes were found, indicating that all human genotypes are susceptible to vCJD prions (Ironside et al., 2006). Whether all genotypes will manifest disease like the M/M cases, or whether a silent carrier state exists is still a matter of debate, as is the possibility that other genotypes might have significantly longer incubation periods, possibly leading to further waves of vCJD in the years to come.

The future

Important questions still need to be resolved for the future. In particular, it is not clear how a global BSE scenario will evolve. While there is still the option that classical BSE can and will be eradicated, this is uncertain for other animal TSEs if they may be of spontaneous origin, or may be transmitted via the environment. In terms of food safety, the evidence in animal TSEs such as scrapie in sheep and goats, or chronic wasting disease in North American cervids, is not conclusive as whether or not these pose a risk.
to human health. Here the precautionary principle can be taken into account, however, history shows no evidence that these TSEs form a food safety risk. More recently, it is not clear what the newly identified “atypical” prion strains mean to risk for humans and animals, and these forms include not only atypical BSE but also atypical scrapie in small ruminants (Baron et al., 2007). Finally, it is not predictable whether and of what size another wave of vCJD in the UK will emerge, as it is not predictable yet what the numbers of secondary human-to-human transmissions of vCJD in the medical setting and by blood will be. The presently available fragmentary scientific answers make it clear that it is yet much too early for complacency and closing the TSE storybook.

Conclusions

The decline of BSE in the EU, as shown by surveillance data, demonstrates that the measures taken, based on numerous science-based risk assessments, were and are effective to control the risk; they are also proportionate to the risk. It also indicates that this was and is the correct way of preventing further spread of BSE and at the same time protecting human health.

While BSE declines in the EU15 and some third countries, this is not clear elsewhere: the peak of BSE incidence in the new EU-MS occurs some time after the peak in the EU15, resulting in some uncertainty about the future situation in the new EU-MS. Moreover, data from the new EU-MS, Japan, and North America indicate a second wave of infections around the year 2000 that should be closely followed.

For BSE detection, active surveillance remains the most effective, although expensive tool; but it may be sustainable if better targeted to risk groups. In all cases, we need full denominators, e.g., breakdown of tests by age and category, and structure of the cattle population; this allows comparisons between specific or adjusted rates rather than using crude rates. In particular, the analysis of birth cohorts is essential to get an idea of the evolution of the risk. Unfortunately, the level of surveillance differs between countries, hence may give different, not to say less reliable or no conclusive results. Lack of good surveillance data makes it difficult to prove the correctness of the assessment and to predict the future development of the risk.

Enhanced surveillance succeeded in the new detection of “atypical” prion strains. Our current very limited knowledge results in uncertainty about their public health risk.
Moreover, if some prion strains occur spontaneously as sporadic disease, their eradication will become impossible.

From an animal health point of view, controlling contagious diseases in their early stages is easier and less expensive for the international community. This is in line with the general approach of DG SANCO in their animal health strategy for 2007—2013 entitled “Prevention is better than cure”. This also indicates the future approach of the EU, to spend on training and awareness on food safety issues and prevention outside Europe rather than on controls. Globalisation in general also necessitates global thinking in terms of food safety. There is a need to continuously carry out risk assessment based on quickly evolving scientific findings in this field. EFSA, in collaboration with the EC and global partners such as the OIE, FAO and WHO, continues to play a major role in this, including offering support (e.g. training, technical support, explanation) to risk assessors in third countries.

References


EU-wide baseline studies: achievements and difficulties faced

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With the purpose of providing the scientific basis for setting targets for Salmonella in food-producing animals, several EU-wide baseline studies to determine the prevalence of Salmonella have been organised at the European Community level. The studies in laying hens and broilers are completed and reported. The achievements and difficulties faced are the main focus of this paper. The results support that poultry products, and table eggs in particular, are important sources of human salmonellosis in EU. However, they also illustrate that with effective surveillance and control efforts, as implemented in several Member States, the prevalence, and thus the associated human infections, can be reduced significantly.

Introduction

Salmonella is an important cause of foodborne disease in humans throughout the world and is a significant cause of morbidity, mortality and economic loss (Adak, Long, & O'Brien, 2002; Mead et al., 1999; Roberts & Sackett 1994; Schroeder et al., 2005; Voetsch et al., 2004). Illness can range from a mild to severe gastroenteritis and in some people, invasive disease, which can be fatal. Long term sequelae such as reactive arthritis can also result from Salmonella infections.

In 2006, the reported number of cases and incidence of human salmonellosis in the EU were 160,649 cases corresponding to 34.6 cases per 100,000 inhabitants (EFSA, 2007a). Salmonellosis continues to be the second ranking zoonosis in EU after campylobacteriosis, but the incidence has decreased over the past years, and in the last three years this decrease has been statistically significant. Of the more than 2500 serovars of Salmonella enterica, S. Enteritidis is the most frequently reported serovar causing more than 50% of the reported human Salmonella infections in the EU. The second most reported serovar is Salmonella Typhimurium being reported from around 13% of the human cases.

S. Enteritidis is also the serovar most often associated with eggborne infections in human, and eggs are considered the predominant source of human salmonellosis in Europe as well as many other countries worldwide (Schroeder et al., 2005; Thorns, 2000). In the EU, eggs and egg products were the most frequently reported source of foodborne outbreaks caused by Salmonella in 2006 (EFSA, 2007a).

The emergence of S. Enteritidis over the past 20–25 years in both table-egg laying hens and humans has been explained by the combination of two main factors: the extraordinary epidemiology of S. Enteritidis infections in laying hens and the centralised rearing of breeding stock (Thorns, 2000). In contrast to most other zoonotic Salmonella serovars, S. Enteritidis has been shown to be able to cause a lifelong colonisation of the peri-reproductive tissue of the laying hens (Humphrey, 1999). This may lead to colonisation of the egg content during the formation of the egg in the reproductive tract. Due to this ability of vertical transmission, parent stock can transmit the infection to their progeny and laying hens can infect the content of eggs produced for consumption. A few other serovars (e.g. S. Typhimurium) may also be transmitted vertically although this occurs more seldom (EFSA, 2005).

For Salmonella spp. in general, the usual route of infection is through horizontal transmission. Infected or contaminated animals will carry Salmonella in the faeces or on the skin, and for slaughter animals, the contamination or cross-contamination of carcasses is basically a question of redistributing the Salmonella bacteria from the positive animals during slaughter and further processing. The epidemiology of Salmonella at the slaughterhouse level is, therefore, primarily due to direct or indirect faecal contamination of live animals or carcasses (D’Aoust, 1989).

The EU Zoonoses Regulation (EC) No 2160/2003 requires MSs to take effective measures to detect and control Salmonella of public health significance in specified

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doi:10.1016/j.tifs.2008.06.008
animal species at all relevant stages of production. For this purpose, Community targets shall be established for the reduction of *Salmonella* in certain animal populations. MSs aim to meet these targets through a National Control Programme (NCP). MSs are also required to report all monitoring results once a year to a web-database administered by the European Food Safety Authority (EFSA) (Directive 2003/99/EC). In order to provide the scientific basis for setting the *Salmonella* targets, several European Union-wide baseline studies were organised at the European Community level.

**EU-wide baseline studies**  
The overall objectives of the baseline studies are to obtain comparable prevalence estimates within the Community in order to provide a scientific basis for setting targets for the prevalence of *Salmonella* in specified parts of the food-animal production. Until now seven EU-wide baseline studies have been planned:

- *Salmonella* in laying hens in the table-egg production (1 October, 2004—30 September, 2005),
- *Salmonella* in broilers (1 October, 2005—30 September, 2006),
- *Salmonella* in slaughter pigs (1 October, 2006—30 September, 2007),
- *Salmonella* in turkeys (1 October, 2006—30 September, 2007),
- *Campylobacter* and *Salmonella* in broiler carcasses (1 January, 2008—31 December, 2008),
- *Salmonella* and MRSA\(^1\) in breeding pigs (1 January, 2008—31 December, 2008),

The studies on *Salmonella* in table-egg layers and broilers have been completed and reported, and the results, achievements and difficulties faced are the main focus for the discussions in this paper. For breeding flocks in the table-egg and broiler production, the target for the *Salmonella* prevalence was set on the basis of the 2004 results from the minimum required monitoring programmes already in place,\(^2\) i.e. no additional baseline study was conducted. The summary of the survey results is contained in the EU Report on *Salmonella* in breeding flocks.\(^3\) Regulation (EC) No 1003/2005 sets a target for breeding flocks to ensure that not more than 1% of breeding flocks with more than 250 birds remain positive for *Salmonella* serovars of human health significance by end of 2009. The prevalence target covers the following five serovars: *S. Enteritidis*, *S. Typhimurium*, *S. Infantis*, *S. Virchow* and *S. Hadar*, which were the five most frequently reported serotypes in human salmonellosis at the time of the target setting.\(^4\)

**Baseline studies in table-egg layers and broilers**  
The specific objectives for the baseline studies in laying hens and broilers were:

- to estimate the holding\(^5\) prevalence of *Salmonella*,
- to estimate the flock\(^6\) prevalence of *Salmonella* (broilers only),
- to estimate the holding or flock prevalence of *S. Enteritidis* and *S. Typhimurium*,
- to investigate the serovar distribution and determine the most frequently occurring serovars,
- to investigate the effect of potential factors influencing the occurrence of *Salmonella*, e.g. *Salmonella*-vaccination status, antimicrobial treatment, and production type,
- to evaluate the sampling designs.

**Sampling designs**  
The laying hen study was conducted on commercial large-scale laying hen holdings with at least 1000 laying hens on the holding. Samples were taken from one flock per each randomly selected holding during the last nine weeks prior to depopulation. Flocks were sampled by taking five faeces samples, each consisting of 200–300 g of pooled faecal material, and two dust samples. In floor-producing units and free-range flocks, five pairs of boot swabs (or sock samples which are considered equivalent) replaced the five pooled faecal samples.

The study in broilers was conducted on commercial broiler holdings with at least 5000 broilers in the holding. From each randomly selected holding, in principle one flock was sampled within the three weeks before slaughter. Up to four flocks were sampled, if the total number of flocks to be sampled per MS could not be reached by sampling only one flock per holding. Eight MSs did only sample one flock per holding. Flocks were sampled by taking a total of five pairs of boot swabs in five equal sectors of the flock area.

In both studies, the number of holdings to be sampled was stratified according to the holding size ensuring that a certain number of holdings in different size categories was sampled. All samples were collected by the Competent Authority and all diagnostic testings were performed by the

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1 Methicillin-resistant *Staphylococcus aureus*.
2 According to Directive 92/177.
5 A facility used for the rearing or keeping of poultry (broilers or laying hens).
6 All poultry of the same health status kept on the same premises or in the same enclosure and constituting a single epidemiological unit. In housed poultry this will include all birds sharing the same airspace. One holding can house several flocks.
National Reference Laboratory or another likewise authorised laboratory. All Salmonella isolates were serotyped, whereas phage typing and antimicrobial-susceptibility testing were done on a voluntary basis. For quality assurance, a proportion of the typable strains and of the non-tybable isolates was sent to the Community Reference Laboratory for Salmonella in Bilthoven, The Netherlands. For a more detailed description of the sampling and diagnostic procedures, readers are referred to the technical specifications.

Each MS reported the results of the studies as well as additional information relating to the sampled holdings and flocks to the European Commission that forwarded the data to EFSA after some initial steps of data validation. For each dataset, EFSA applied a set of data exclusion criteria in order to identify non-valid and non-plausible information. All together approximately 6% of the laying hen holdings and 3% of the broiler flocks were excluded using the criteria set by EFSA. This resulted in two cleaned and validated datasets comprising of data from 5310 holdings of table-egg layers from 24 MS and Norway, and 6325 holdings and 7440 flocks of broilers from 23 MS and Norway. All prevalence estimations and statistical analyses were performed by EFSA. A more detailed description of the methods applied including the exclusion criteria can be found in the individual baseline study reports (EFSA, 2007b; EFSA, 2007c; EFSA, 2007d).

Results and difficulties encountered

Laying hens

Overall, Salmonella was detected in 1486 laying hen holdings in the EU. The MS-specific holding prevalences ranged from a minimum of 0% in Luxembourg and Sweden to a maximum of 79.5% in Portugal meaning that both the MS-specific prevalences and the EU weighted mean must be regarded as a minimum. Two factors contributed to this underestimation. First of all, since flocks on the same holding may have different Salmonella status, sampling of a negative flock on holdings where one or more positive flocks were present (but not sampled) resulted in a false negative diagnosis of the holdings. Secondly, flocks from smaller holdings were more likely to be sampled than flocks from larger holdings, and since the results of the study indicate that larger holdings are more likely to be positive, this contributed further to the underestimation of the holding prevalence.

Despite this underestimation, the prevalences were for most MSs found to be considerably higher than the regular monitoring results as reported in the Community Report 2004 (EFSA, 2006a). There are many plausible reasons for this observation. The study design applied in the baseline study, where dust samples were taken, is likely to be more sensitive. Also, the layer flocks were investigated at the end of their production period, where the within-flock prevalence may be higher, whereas the regular monitoring may cover different age groups and different stages of the production. Some national monitoring programmes may also be targeted to specific serovars, particularly S. Enteritidis, and/or include holdings with less than 1000 birds. Both will lead to a lower observed prevalence. Finally, the fact that the Competent Authorities and the National Reference Laboratories were responsible for sampling and testing may have contributed to the detection of more flocks than expected when compared to the results of the regular monitoring. Still, it is noteworthy that additional infected flocks were unexpectedly identified in some MSs with very active surveillance and control programmes (Fig. 2).

In order to investigate the potential effect of various factors on the occurrence of Salmonella in layers, regression analyses were performed. As risk factors for Salmonella positivity may depend on the serovar in question, the analyses were performed separately for S. Enteritidis, S. Typhimurium and for serovars other than Salmonella Enteritidis and Typhimurium. Furthermore, the MSs were divided into three S. Enteritidis prevalence groups, which were analysed separately: a low (<2.5%), intermediate (2.5–15%) and high (>15%) prevalence group (Fig. 1). In the low prevalence group, there were only very few S. Enteritidis positive flocks and a regression analysis was not attempted.

Factors examined in the analyses included: type of sample (dust or faeces), age of hens in weeks, production type (cage, barn, free-range standard or organic), vaccination status (unvaccinated, vaccinated against S. Enteritidis, vaccinated against non-S. Enteritidis), antimicrobial treatment in the two weeks prior to sampling (yes or no), mixed or homogeneous age flock, month of sampling, and days from sampling to laboratory testing. Several variables describing the size of the holdings were recorded: number of hens in the holding, number of flocks in the holding, and number of hens in the

flocks. These were all found to be highly correlated and in addition strongly associated with the production type. Almost two-thirds of the holdings in the study were of the cage production type, and most of these belonged to the largest size categories. Organic flocks had on average the smallest size, whereas the barn and the free-range flocks were of low to medium size. This meant that any effect of production type and holding size variables could not be disentangled in the analyses, and it was consequently decided only to include the production type as a potential risk factor.

Cage production was found to be associated with a higher risk of *Salmonella* positivity than the other production types, but as explained above this effect may be associated with larger flock and/or holding sizes, as well as higher animal density. An effect of parent stock and/or hatchery has also been described (Skov, Angen, Chrieël, Olsen, & Bisgaard, 1999) and since producers of day-old chicks often supply only a single type of production, *Salmonella* problems in the top of a breeding pyramid system that mainly supplies the cage production will result in an effect linked to this production type only.

The analysis indicated that an increase in the age of the hens present in the flock at sampling was related to a higher observed prevalence of *S. Enteritidis* at both the EU-level and in the MS group with the highest prevalence. This observation supports the point raised above that the within-flock prevalence is likely to be higher in flocks reaching the end of their production period. An association of increased age and higher prevalence was also seen for serovars other than *S. Enteritidis* and *S. Typhimurium*, but not for *S. Typhimurium*.

When investigating the effect of vaccination only data from those countries with a voluntary vaccination policy was included i.e. data from countries, where vaccination is either prohibited or mandatory was not included and the interpretation of the results should be seen in this light. Overall, vaccination appeared to protect against *Salmonella* infections in MSs with a high *Salmonella* prevalence. However, in MSs with an intermediate *S. Enteritidis* prevalence, vaccination seemed less important for the *S. Enteritidis* status. The observed protective effect of vaccination may, therefore, also be explained by a general increased awareness and implementation of preventive measures in vaccinated flocks.

There were indications that factors associated with *Salmonella* prevalence may depend on the epidemiology of the *Salmonella* serovar (e.g. mode of transmission). While *S. Enteritidis* and *S. Typhimurium* showed no evidence of seasonal variation, the occurrence of other serovars was found to be associated with the month of sampling with a peak in the autumn months. This might be explained by an increased occurrence of these other serovars in feed during these months (Angen, Skov, Chrieël, Agger, & Bisgaard, 1996), but a study over several years is required if a true seasonal effect shall be demonstrated.

Finally, dust samples were more likely to be positive than faeces samples in all analyses. This suggests that sampling of dust increases the sensitivity of *Salmonella* detection in a laying flock environment, and as discussed above this may in part explain the observed differences between the results of the baseline study and the national monitoring reported in the Community report.
In contrast to the laying hen study, it was possible to estimate the flock prevalence in the broiler study, because the majority of the MS had sampled more than only one flock per holding. This measure was preferred over the holding prevalence as the flock is the more relevant sampling unit from an epidemiological perspective. However, the flock prevalence estimation could not be done without certain assumptions that are difficult to verify. These include the assumptions that flocks at the same holding, in the same country, at the same time have similar observed prevalences and that the between-holding variability of observed flock prevalence is comparable across countries i.e. either the same or increases proportionally to the level of infection. Considering some of the systematic differences observed between MSs in factors that potentially influence the *Salmonella* prevalence (e.g. productions types, vaccination policies, and control efforts), this may not always be the case. Still, the approach was considered reasonably valid and more reliable than the holding prevalence for the reasons explained for the layer study above. For future studies in both broilers and layers, it is recommended to apply a multi-level sampling design, where flocks to be sampled are picked randomly among all the holdings in the MS.

*Salmonella* was detected in 1448 broiler flocks corresponding to a Community weighted observed flock prevalence of 23.7%. Again, the EU weighted mean makes little sense, as the observed flock prevalence differed considerably between MSs, from a minimum of 0% in Sweden to a maximum of 68.2% in Hungary (Fig. 3). High prevalences were also observed for Poland (58.2%), Portugal (43.5%) and Spain (41.2%). A comparison of the MS-specific prevalences with those reported in the Community Report for 2005 (EFSA, 2006b), showed that these were either comparable or considerably higher (Fig. 4), which may be explained by a more sensitive sampling design and/or an increased test sensitivity of the analytical method applied in the baseline study. Other factors as mentioned above for the laying hen study may also have contributed to these differences.

As for the laying hens, *S. Enteritidis* was the most frequently reported serovar in broiler flocks followed by *S. Infantis* (Table 1). Together, these two serovars were isolated from more than half of all *Salmonella* positive flocks.

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<td>295</td>
</tr>
<tr>
<td><em>Salmonella</em> Mbandaka</td>
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<tr>
<td><em>Salmonella</em> Ohio</td>
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<tr>
<td><em>Salmonella</em> Bredeney</td>
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<tr>
<td><em>Salmonella</em> Tennessee</td>
<td>28</td>
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<td>5</td>
</tr>
<tr>
<td>Other serovars</td>
<td>—</td>
<td>—</td>
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</tr>
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</table>

* Data received from the European Centre of Disease Prevention and Control (ECDC) by the EFSA Zoonosis Collaborating Centre.
Other frequent serovars included *S. Mbandaka* (8%), *S. Typhimurium* (5%) and *S. Hadar* (4%). Although *S. Virchow* was found in only 2.1% of all *Salmonella* positive broiler flocks, it was reported by 11 MS indicating that it is among the more widely spread serovars throughout the EU.

Due to the observed geographical variation in the serovar distribution in the EU, a formal spatial analysis was performed. The analysis indicated that particularly *S. Mbandaka* and *S. Infantis* were clustered to Ireland and Hungary, respectively. Relative risks (RR) of 48.3 and 20.5 suggest that broiler flocks in these areas are 48 and 20 times more likely to become infected with *S. mbandaka* and *S. Infantis*, respectively, than broiler flocks outside these regions. Although 17 MSs reported *S. Enteritidis*, Portugal and Spain were identified as the most likely cluster for this serovar (RR = 6.2). The most likely cluster for *S. Typhimurium* included Hungary, Slovakia and Poland (RR = 9.6). The area including Poland was identified as the most likely cluster for *S. Hadar*.

The spatial analysis was done at the country-level, as this was the only geographical information available. In future studies, it could be of interest to include more detailed spatial information (e.g. region, community or coordinates of the individual holding) in order to obtain a more detailed picture of the distribution of certain serovars. Although factors at the country-level may explain the clusters observed, other factors not bound by country borders (e.g. climate and topography) could be of importance. These might be identified in a spatial analysis performed on for instance the holding level.

The risk factor analyses were done separately for *S. Enteritidis*, *S. Infantis* and the group of other serovars. The following factors were investigated: month of sampling, days from sampling to laboratory testing, number of flocks in holding, number of animals in flock, age of broilers in weeks, production type (conventional, free-range standard or organic), number of cycles, and antimicrobial treatment in the two weeks prior to sampling (yes or no).

Generally, only a few of the investigated factors were found to be associated with *Salmonella* infection in broiler flocks. This is probably due to the fact that many of the known factors important for *Salmonella* control in broilers (e.g. biosecurity measures, feed management and status of parent flocks) were not included (see e.g. Chriél, Stryhn, & Dauphin, 1999; Davies & Wray, 1995; Davies & Wray, 1996; Gradel & Rattenborg, 2003; Rose et al., 1999). The results should, therefore, be interpreted with care, as the identified risk factors may not be true causal or the most important risk factors.

The month of sampling was associated with positivity in all three analyses with the highest risk occurring during the
Role of table eggs and broiler meat in human salmonellosis

By comparing subtypes of Salmonella isolates from different sources (e.g. animals and food) with those isolated from humans, it is possible to make inference about the most important sources of human infections (Hald, Vose, Wegener, & Koupee 2004). The subtyping methods applied should be definitive and discriminatory and may besides serotyping include phage typing, antimicrobial-susceptibility testing and genotyping e.g. pulsed-field gel electrophoresis and sequence-based subtyping.

In the baseline studies, phage typing of S. Enteritidis and S. Typhimurium and antimicrobial-susceptibility testing were done on a voluntary basis and subtyping results were, therefore, only reported from a subset of MSs and in some of these only a few isolates were recovered or typed. Also, the lack of harmonised methods for antimicrobial-susceptibility testing made comparison between MSs difficult. Finally, phage type distributions in humans were only available for a fraction of the MSs. Taken together, these factors complicated a thorough analysis of the subtypes in humans, laying hens and broilers with the purpose of estimating the role of table eggs and broiler meat as sources of human infections. Still, some overall conclusions based on a comparison of the data available could be made and are discussed below. However, before addressing these, one of the recommendations pointed out in the baseline study reports should be emphasised — namely that phage typing and antimicrobial-susceptibility testing should be mandatory in future baseline studies. Besides being useful for tracking sources of human infections, antimicrobial-susceptibility testing is important in order to follow the trends and distribution of resistant Salmonella subtypes, particularly those developing or acquiring resistance to important human therapeutic antimicrobials such as fluorquinolones and third generations cephalosporins (WHO, 2007).

Eggs are commonly considered the predominant source of human salmonellosis in many parts of the world including in Europe (Thorns, 2000). This was supported by the results of the baseline study in laying hen holdings, where S. Enteritidis was demonstrated to occur widely in the laying hen production in many MSs. However, S. Enteritidis was also the most frequently isolated serovar in broilers and S. Enteritidis phage types commonly found in human cases were also reported from the broiler flocks (Table 1).

A further comparison of the Salmonella serovar and phage types from broilers, laying hens and humans suggest that although the majority of human S. Enteritidis infections is considered to be caused by contaminated eggs, a certain proportion of human infections occurring from broilers cannot be excluded. This role of broiler meat as a source of human infections is likely to be more important in countries with a high S. Enteritidis prevalence in broiler flocks.
S. Typhimurium was the second ranking serovar as a cause of human infections in the EU and responsible for approximately 10% of the reported cases. In the two baseline studies, S. Typhimurium was found in broilers and in laying hens in the majority of MSs and generally the same MSs reported S. Typhimurium positive flocks in both studies. However, the occurrence of S. Typhimurium was much lower than that of S. Enteritidis. S. Typhimurium is a serovar, which is often found also in other animal species assumed to form a source of human Salmonella infections, such as pigs and cattle. In fact, S. typhimurium is reported to be the dominant Salmonella serovar in pigs and cattle in the EU (EFSA, 2007a). Therefore, it is likely that only a minor part of the human S. Typhimurium infections may be attributed to broiler meat and table eggs.

For serovars other than S. Enteritidis and S. Typhimurium, the most commonly reported serovars found in human infections at the EU-level include S. Infantis, S. Bovismorbificans, S. Hadar, S. Virchow, S. Derby, S. Newport, S. Anatum and S. Goldcoast. While S. Bovismorbificans and S. Goldcoast were only isolated from a single flock in the broiler study, S. Infantis, S. Hadar, S. Virchow, S. Anatum, and to some extent S. Newport were relatively often detected from the broiler flocks. This suggests that broilers and broiler meat may be a relevant source of these serovars for humans. Still, more detailed knowledge of the serovar distribution in other food animals species is needed to quantify the relative role of broiler meat.

Conclusion and achievements

In conclusion, the results of the baseline study in laying hens show that S. enterica, particularly S. Enteritidis, occurs widely in the commercial large-scale laying hen production in the EU, although at varying levels in different MSs, which strongly indicates that table eggs continue to be an important — and probably the most important — source of human salmonellosis in EU. The baseline study in broilers supports the role of broiler meat as an important source of human Salmonella infections, particularly in MSs with a high Salmonella prevalence in broilers. Human salmonellosis caused by broiler meat is not related to a specific serovar in contrast to those due to eggs or egg products.

The baseline studies have achieved their major objectives: to provide comparable prevalence estimates for Salmonella in laying hens and broilers to be used for formulating EU-targets. For table-egg layers, the targets have been set to maximum 2% flocks positive for S. Enteritidis and S. Typhimurium, or a certain percent reduction dependent on the baseline prevalence. The target should be reached by 2011. In addition, all MSs should by January 2008 have implemented a minimum surveillance programme in laying hens. In broilers flocks, the reduction target is a reduction of the maximum percentage of flocks of broilers remaining positive for S. Enteritidis and S. Typhimurium to 1% or less before 2012. A minimum surveillance programme in broilers should be implemented before January 2009. The studies also identified differences between results from the baseline studies and regular monitoring results, emphasising the need for harmonisation of monitoring programmes in the EU.

The baseline studies provided insight into some of the factors that may be associated with Salmonella status e.g. production type, vaccination status, and age of hens/broilers. Still, the studies were at first hand not designed for conducting risk factor analyses and the interpretations of the results were not without problems.

Finally, the baseline studies confirmed that poultry products — and in particular table eggs — continue to be important sources of human salmonellosis in EU, but also that effective surveillance and control efforts, such as top-down eradication and heat treatment of eggs from infected layer flocks or vaccination of breeder and/or layer flocks, as implemented in several MS, can significantly reduce the prevalence in laying hens and broiler flocks, and thus the associated human infections (Cogan & Humphrey, 2003; Korsgaard, Madsen, Feld, Mygind & Hald, in press; Poirier et al., 2007; Wegener et al., 2003).

References


Notes:


This article provides an overview of consumer risk assessment and aims to show how pesticide residues data from field trials (supervised residue trials) are combined with dietary consumption data to assess risk to consumers. Models used for the calculation of dietary exposure are based on the premise that intake is a function of the concentration of pesticide in food and the amount of food consumed. The sources of data needed to inform these models are examined and some consideration is given to the future of risk assessment modelling. The emphasis is on current EU procedures.

Dietary intake

Pesticides are an integral part of modern food production and consumers are aware that they ingest pesticides with their food. One of the key issues that interests both regulators and consumers is the question of how much pesticide is consumed. The amount of pesticide consumed is termed dietary intake, or simply intake, and the models used in its calculation are the subject of this article. As the tool for quantifying risk the models are examined and some consideration is given to the future of risk assessment modelling. The emphasis is on current EU procedures.

Risk assessment

In the first instance it is necessary to define the identity of the pesticide residue for which the risk assessment is to be performed, that is to set a ‘residue definition’. All metabolites and degradation products of toxicological concern are considered for inclusion in the residue definition used for risk assessment (European Commission, 1997a). If the residue definition is not specified accurately disagreements between regulatory agencies can occur when decisions are made at the risk management stage.

The toxicological reference dose is an estimate of the amount of pesticide that can be ingested without appreciable health risk on the basis of all known facts at the time of the evaluation (JMPR, 2002). There are two types of reference dose: the Acute Reference Dose (ARID) which is the amount that can be ingested over a short period of time, usually one meal or 1 day, and the Acceptable Daily Intake (ADI) which is the amount that can be ingested daily, over a lifetime. Both reference doses are expressed on a body

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weight basis and normally incorporate an uncertainty factor (also known as an assessment factor) of 100 to account for inter-species variability (between the test animal species and humans) and intra-species variability (between the individuals within the human population) (Institute for Environment and Health, 2004).

For a given pesticide the acceptability of a level of dietary intake is calculated by comparing it to the appropriate reference dose (either the ARfD or the ADI). Approval for use of a pesticide is given if the dietary intake is equal to or below the toxicological reference dose. This means that the risk characterisation stage of a true risk assessment (Organisation for Economic Co-operation and Development, 2003) is not required as approval for use of a pesticide is only given if it has been shown that the risk to consumers is negligible. Intake is often expressed simply as a percentage of a reference dose; a calculated intake above 100% of the reference dose is therefore considered an unacceptable risk.

Modelling intake

The models used for the calculation of intake are based on the premise that intake is a function of the concentration of pesticide in food (usually referred to as the residue level) and the amount of food consumed. The data needed to inform the models come from two sources: residue levels from supervised field residue trials (often simply referred to as residue trials) and consumption data from food consumption surveys, which will be looked at later.

Residue trials

Residue trials are of central importance to the models and they are also required to support the approval of a pesticide for a use on a given crop. In the EU eight trials are required for a major crop such as apples and four for a minor crop such as spinach (European Commission, 1997b). In residue trials crops are grown according to a specified agricultural practice (termed Good Agricultural Practice, GAP). The agricultural practice used must be the same as that on the label or notice of approval attached to the approved product. This is not always straightforward and it is known that residue levels are often higher in crops grown in trials than crops grown in normal agricultural practice. This is largely due to the fact that the GAP represents a worst-case scenario: it involves application of the maximum possible dose and the minimum interval between the last application and harvest, both factors which tend to lead to higher residues in the edible parts of crops.

However trials are not a type of controlled laboratory experiment but accurately simulate conditions in normal agricultural or horticultural practice. For instance it is normal for there to be an uneven distribution of residues on the crop due to factors such as variation in the size of fruit or in the degree of cover offered by foliage. When sampling the aim is to gather a number of individual items which, when combined together, represent the average residue level having accounted for natural variation. This principle continues through the sampling process: from the crop in the field to the field sample, to the laboratory sample, to the analytical sample.

A full set of residue trials data are important and are utilised in the setting of MRLs (European Commission, 1997c) but two critical values are taken from the full set; the highest residue (HR) and the median residue or STMR (Supervised Trial Median Residue). A complete set of trials is not always required as for certain crops it is possible to make use of an agreed set of extrapolations (European Commission, 2008) such that data on one crop can be used to support use on a range of crops.

Modelling exposure

The concentration of pesticide in food is normally taken to be either the highest residue level, HR (in the assessment of acute risk) or the median residue level, STMR (in the assessment of chronic risk) and the amount of food consumed is denoted by F.

Dietary intake (of pesticide) =

\[
\frac{\text{Concentration of pesticide in food} \times \text{Food consumed}}{\text{Body weight}}
\]

This simple equation is used to calculate intake but since exposure is relative to body weight there is an additional term in each of the models:

Exposure (to pesticides in the diet) =

\[
\frac{\text{Concentration of pesticide in food} \times \text{Food consumed}}{\text{Body weight}}
\]

The mean body weight for all consumers or a specific consumer group is used. If individual body weights were used the resulting units would not allow certain complexities of the calculation (described below) to be performed. Beyond this level of simplicity the models used to assess acute and chronic exposure differ.

The acute model

This model is used to calculate short-term intake and is known by the abbreviation NESTI (National Estimate of Short Term Intake) or IESTI (where the first I stands for International). In its basic form this model is the simpler of the two:

\[
\text{Intake} = \frac{\text{HR} \times \text{F}}{100}
\]

The HR is used as it is possible for a consumer to eat an item in a single sitting or day that contains residues at the highest level known to occur. The HR may be subject to modification on the basis of processing studies (HR becomes HR – P) which is a more refined estimate of the residue level in food at the point of consumption. Processing studies involving common household or industrial practices (e.g. cooking, canning) may show a significant decrease, or
Acute intake

\[ \text{Acute intake} = \frac{(U \times HR \times v) + (F - U) \times HR}{\text{Mean body weight}} \]

Where \( v \), the variability factor, is defined as:

\[ v = \frac{97.5\text{th percentile residue level (in a batch)}}{\text{Average residue (in a batch)}} \]

and \( U \) is the weight of a single item of the food in question (termed the ‘commodity unit weight’).

It is possible for there to be a 10-fold difference between the 97.5th percentile residue level and the average level; in other words for \( v \) to equal 10.

Variability

What relevance does variability have to consumers?

Consider a medium sized commodity such as an apple, the commodity unit weight of an apple (\( U \)) by some estimates is 112 g (\( U \) (apple) = 112 g). Now consider eating the apple: if the residue level in the apple happened to be high due to natural variation (i.e. if the apple was ‘hot’), its contribution to intake would be disproportionately large. Compare this to a small commodity such as a pea: even if several ‘hot’ peas are consumed together the intake tends towards the average because such commodities are generally eaten in multiples at a single sitting.

Whereas unit-to-unit variability has a greater impact on intake for larger commodities Table 1 shows that the value of \( v \) is inversely related to size: for melon \( v = 5 \) and for apples, pears and citrus fruit such as oranges \( v = 7 \).

The value of \( U \) for an apple, given above, is that used in the UK (Pesticides Safety Directorate, 2005) but different European Member States can have different values and since there is no standard means of determining \( U \) for a given fruit or vegetable such discrepancies are frequent. If the value of \( U \) happens to be below one of the thresholds it can mean that \( v \) can take a different value or does not apply at all. As a result, while there is agreement that variability must be accounted for, in practice, consistency is not assured.

Accounting for variability

Calculations of acute intake commonly fall into three different cases. Case 1 (e.g. peas) where \( U \) is much less than \( F \) has already been illustrated. Cases 2a and 2b apply to commodities of such a size that variability must be accounted for. This is done by assuming that a proportion of the total amount of a particular food consumed in a day contains a higher amount of pesticide due to variability. In case 2a the unit size is less than \( F \), for example apples which can be eaten in multiples in a single day. The variability factor is applied to the first apple (the term \( U \times HR \times v \)) and the remaining amount (\( F - U \)) is not considered to be ‘hot’. The assumption is that it is unlikely that one ‘hot’ apple would be followed by another.

In the other case of interest here, case 2b, the unit size is greater than \( F \); for example melon which is so large that only part can be consumed in a single sitting. The variability factor is applied to the whole portion (the term \( U \times HR \times v \)). The difference is if one selects a ‘hot’ melon the whole of that portion, and therefore the whole of \( F \), is ‘hot’.

Assumptions of the acute model

The acute model is based on the assumptions that there is no loss of residues on storage or transport, and all produce is treated. These are worst-case or high-end type assumptions and are part of the internationally agreed approach (FAO, 2002). The former can be unrealistic and lead to an overestimation of intake and the latter is used as it is possible for an individual to obtain all produce from one source such as a local farm or a brand to which they are loyal.

The chronic model

This model is used to calculate intake over the long term (a lifetime) and is known by the abbreviation NEDI (National Estimate of Dietary Intake) or IEDI (where the first I stands for International). The chronic model uses the STMR from the residue trials and the amount of food consumed, although the value of \( F \) is calculated differently (explained below):

\[ \text{Intake} = \text{STMR} \times F. \]

This simplifies the model and makes sense as, calculated over a long period of time, residues experienced by the consumer tend towards the average. Unit-to-unit variation is averaged out for the same reason and so \( v \) does not need to be considered. However there is a complexity in the chronic model which arises from the need to consider the

<table>
<thead>
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<th>Table 1. Variability factors</th>
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<tr>
<td><strong>Commodity unit size</strong></td>
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<tr>
<td>Large (&gt;250 g)</td>
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<tr>
<td>Medium (25–250 g)</td>
</tr>
<tr>
<td>Treated with</td>
</tr>
<tr>
<td>soil applied granular pesticide</td>
</tr>
<tr>
<td>Treated with</td>
</tr>
<tr>
<td>non-granular, non-soil</td>
</tr>
<tr>
<td>applied pesticides</td>
</tr>
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</table>

Weights given in column 1 define the weight of \( U \) for which the given variability factor applies.
sum of all the intakes from all commodities that may be consumed over a long period of time. This sum is known as the Total NEDI and for its calculation detailed information about the dietary behaviour of consumers is required.

Food consumption data

Food consumption studies are the other source of data which form the second pillar supporting the intake models. The many ways of collecting food consumption data are sub-divided into two groups: individual- and population-based methods. The UK has for many years used an individual-based method; a type of dietary survey based on a weighed diary of food consumed over 7 consecutive days. Other countries have used a population-based method known as the Food Balance Sheet which relies on statistics on a country’s food production, imports and exports. Whichever method is used all countries are confronted with the same difficulties in gathering consumption data. Population-based methods are generally cheaper and easier to keep up to date but they can overestimate consumption (partly because they can’t account for wastage which can be significant for perishable fruit and vegetables) and do not provide any information about acute consumption. It is also possible for population-based methods to underestimate individual consumption; for example the consumption of an exotic fruit in relatively large amounts by a small number of people, would appear to be consumed in small amounts when considered from the point of view of the whole population. Individual-based methods are more complicated and more expensive and their representation of the general population depends very much on the sample size. To understand the challenges posed by both methods three key areas are now examined.

Data collection

a Individual-based methods involve at least some face-to-face contact so the sample size must be limited if the cost is to be acceptable. As a consequence the sample size can be quite low and even lower when seasonality is factored in. This point should not be lost: for commodities that are consumed seasonally, such as strawberries, the surveys don’t actually cover as large a sample as might appear since a good proportion of the survey will have been in the off-peak season. Sample size can vary widely; from less than 100 to in excess of 5000 individuals. When a small sample is extrapolated to the entire population it becomes problematic.

b The potential for bias in any dataset increases as the level of non-response increases. In the last survey of UK adults (2000–2001), 37% of the initial sample refused to take part (Henderson, Gregory, & Swan, 2002), calling into question the validity of the survey. The high level of non-response contributed towards the drive to review how the UK’s dietary survey programme is conducted (Food Standards Agency, 2006).

c The aim of many studies of this nature is principally to understand the nutritional status of the population; they are therefore not designed to meet all the subsequent purposes to which they are put. One consequence is that data are sometimes not available on foods of low nutritional value and yet these foods may be of high concern in terms of pesticide content. Another consequence is that the food groups used in surveys do not always correspond directly with the crop groups in MRL legislation. In some cases the mismatch requires careful interpretation if the data are to remain meaningful.

d It is well known that individual-based methods can underestimate consumption, possibly because people’s behaviour is affected by their involvement in the survey.

Statistical analysis

Once the data have been collected there is further scope for differences to arise out of different statistical treatments of the data. For example in one Member State, when considering rarely eaten foods, the choice of percentile depended on the number of consumption days recorded in the food diary database: for instance where the survey had between 10 and 19 consumption days the 90th percentile is used and where there is less than 10 consumption days the maximum reported value is used (European Food Safety Authority, 2007a).

Methodology

Although a methodology for the calculation of intake has been agreed under the World Health Organisation (FAO, 2002) there are still subtle differences in the methods used in different countries. Such differences can lead to the creation of trade barriers and the need to avoid such problems was one motivation behind the recent project of work undertaken by the European Commission to harmonise MRLs across the EU.

EFSA/PRAPeR (“PRIMO”) model

As part of the harmonisation project the European Food Safety Authority (EFSA) was asked by the EU Commission to evaluate MRLs proposed by Member States. This presented a challenge relating to the possibility that a given MRL could be considered safe in one country, but not in another, due to real or apparent (for the kind of reasons discussed above) differences in food consumption patterns. To deal with this EFSA created a single model to hold all the consumption data from the Member States which is now known as PRIMO (Pesticide Risk Assessment Model) (European Food Safety Authority, 2007a).

In collecting this information EFSA have highlighted one of the many areas of diversity in the EU. It could be
argued that such diversity still exists because there has been no attempt to harmonise the way consumption data are collected and reported. Undoubtedly it is also because it involves two key elements that define cultural differences: language and food.

The PRIMO model contains data on food consumption (F), the estimates of unit weight (U) used in various Member States and the body weight of the population or sub-populations involved in the surveys. The value of U has been shown to vary across a surprisingly wide range: U (apple) ranges from 90 to 182 g, inexplicably U (pineapple) can be as low as 22 g and U (raspberry) as high as 118 g. It is likely that some of these differences are due to interpretation; in the UK for instance U (table grape) is 500 g which could easily be viewed as a mistake however in this instance U is considered to apply to a single bunch of grapes, not a single grape.

With a population of approximately 500 million the average consumer in the EU is difficult to define. Nevertheless the PRIMO model, like the national models on which it is based, is designed to account for the whole population including the average consumer as well as individuals with higher consumption habits. It is therefore necessary to take the food survey data involving hundreds of individuals over a limited number of days and extract a single value of F that is both realistic and representative.

For the acute model a single value is taken from the distribution of the highest daily consumption rates: F is the 97.5th percentile in the distribution. In other words F is taken from the high end of the highest consumption rates recorded by individual consumers over all diary days. However, since the risk management objective is to identify high consumers, only individuals who eat the commodity in question are included in the calculation of F. This ensures the distribution does not include a large number of non-consumers which would have the effect of lowering the final value of F.

Another important aspect of the calculation of F that is often overlooked is how data on foods, consumed in their various processed forms, are converted into amounts of the raw agricultural commodity (RAC). In the case of composite foods, fruitcake, for example, a number of component ingredients may be present in small amounts. A ‘cut-off’ may be defined, such that component ingredients are ignored if they happen to be present below a certain proportion. If all components are included, no matter the proportions at which they are present, this adds a large number of low consumers to the distribution which has the effect of lowering the final value of F.

In the UK the chronic model uses two different values of F; a high level and a mean level. This is a unique feature of the chronic model that was developed in the UK (Day, Rees, & Harris, 1996) and will eventually be incorporated into the PRIMO model. The values of F are taken from the distribution of the average daily consumption rates: the high level F is the 97.5th percentile in the distribution and the mean level F is the 50th percentile. In other words the high level F is taken from the high end of the distribution of averages and the mean level F is taken from the mid-point of the distribution (i.e. it is the average of the average consumption).

There is another major contrast with the acute model: all consumers, including those with zero consumption, are included in the calculation of F. This is justified on the basis that it more accurately represents the dietary behaviour of the whole population over a long period of time.

For a risk assessment involving multiple foods, as mentioned above, the Total NEDI is calculated. To do this the chronic model identifies the two foods that represent the highest contribution to diet (considering the high level F) and uses the high level F for both of them. For all other commodities the model uses the mean level F. This subtlety is based on the premise that over a lifetime an individual can maintain a high level of consumption of some, but not all, foods. This is reasonable as there is a physiological limit to what a person can eat and as a result the technique is thought to give a conservative but realistic estimate of the highest exposure to consumers.

There are a number of other factors that should also be noted when considering chronic consumption. Firstly, the average consumption rate must be determined over a few survey days and yet it is used to represent intake over a lifetime. Such an extrapolation may not be truly valid and so ways to interpret the data to achieve a more realistic appreciation of average consumption have been developed. The second point, closely related to the first, is that chronic assessments are concerned with intakes maintained over a lifetime and yet an individual’s consumption levels change over time. This highlights the inconsistency of considering the chronic risk to a child, for example, based on an assessment of intake that accounts for the child’s consumption rate relative to its body weight as a child, both of which will obviously not remain constant over its lifetime.

**Consumer protection**

It is clear that a higher value of F could be taken from the distribution of consumption rates recorded in the food diary and therefore it may appear that consumers with the most extreme diets are ignored. The approach used is justified as the cut-off gives a conservative but accurate estimate of high level consumption prevalent in the population and it is common for the tail end of distributions to include outliers and misreported values. In addition the model uses several high-end values for the distributions: the highest residue in the acute model, a high variability factor and the high level F. The combination of these three high-end values is considered to
Table 2. Number of pesticide/country/age group scenarios that achieve different levels of protection, after excluding all commodities for which the IESTI exceeds the ARfD

<table>
<thead>
<tr>
<th>% of person-days below the given</th>
<th>Number of pesticide/country/age group scenarios (total = 78)</th>
</tr>
</thead>
<tbody>
<tr>
<td>level of intake</td>
<td>1 × ARfD</td>
</tr>
<tr>
<td>0–90</td>
<td>0</td>
</tr>
<tr>
<td>90–99</td>
<td>0</td>
</tr>
<tr>
<td>99–99.9</td>
<td>9</td>
</tr>
<tr>
<td>99.9–99.99</td>
<td>24</td>
</tr>
<tr>
<td>&gt;99.99</td>
<td>45</td>
</tr>
</tbody>
</table>

The second column from left shows the proportion of scenarios with different percentages of person-days below the ARfD. The third and fourth columns show the same for twice and ten times the ARfD, to give an indication of the magnitude of the intakes that are above the ARfD.

give a result that is at the high end of the real distribution of consumption rates.

Conclusions
The deterministic (point estimate) approach to the calculation of intake, the basis of the acute and chronic models discussed above, has been in use for many years. One reason for its success is that it provides a simple to use tool that gives consistent results and it is clear, with a little investigation, how the result has been calculated (qualities that are greatly appreciated by both regulators and industry). The use of point estimates, such as a single value for F (for acute consumption) taken at a pre-defined percentile (the 97.5th percentile) from a distribution of food consumption data simplifies the calculations involved. However for certain foods, particularly those that are rarely consumed, it is possible for the 97.5th percentile of the total distribution to equal zero and this is one of the reasons that non-consumers are excluded from the calculation. The problem is that it is all too easy to define a ‘non-consumer’ as anyone whose food diary did not include the food item in question over the course of the survey, the implication being that they would never consume the food.

What does this mean for the model’s value as a tool for facilitating the protection of the total population, or to put it another way: how conservative is the acute model with respect to the total population?

This question was recently addressed by the EFSA PPR Panel (European Food Safety Authority, 2007b). In order to approach the question the Panel established the term ‘level of protection’ and defined it as the probability of exceeding the ARfD. The Panel used a probabilistic approach to enable the complete distribution of consumption rates to be used instead of a single value of F. The work of the EFSA Panel showed that for a significant number (24 out of 78) of the pesticide/country/age group scenarios investigated the percentage of person-days where the ARfD is exceeded was estimated to be between 0.01 and 0.1%. In other words between 99.9 and 99.99% of person-days were shown to be below the ARfD. Table 2 shows the results of one set of calculations made by the Panel.

These results also emphasise the importance of the degree to which the ARfD is exceeded: a slight exceedance is unlikely to be toxicologically significant whereas an exceedance of 10 times the ARfD could give rise to a toxicological effect.

One difficulty with these results is that ‘person-days’ cannot be directly related to the total population: it is not possible to say if 0.1% of person-days are above the ARfD then 0.1% of the population experiences intakes above the ARfD on a daily basis. A further difficulty in interpreting results presented in this way is that they do not distinguish between the situation where a small number of people are at risk over a large number of days and that where a large number of people are at risk over a small number of days. As a consequence it is not easy for risk managers to know how to respond to such results.

Risk managers must be able to fully appreciate two values expressed as proportions. First; the probability that an individual’s intake exceeds the ARfD, and second; the proportion of the total population that will experience that probability. These two proportions would make it possible to make a statement such as ‘10% of the population has a 0.1% probability of exceeding the ARfD on any single day’. Such a statement can be readily understood and would, perhaps, allow a response. However, in order to respond risk managers would need to decide on an acceptable:

a proportion of the population that should be protected, and;

b level of probability that the proportion of the population in (a) will be exposed to.

The difficulty, of course, is that implicit in any statement defining the proportion of the population that should be protected is an admission that some individuals within the population are not protected. Clearly this makes it a political decision that is very much outside the risk assessors’ domain.

References
Appendix B, General recommendations for the design, preparation and realization of residue trials.


Exposure to multiple chemicals: when and how to assess the risk from pesticide residues in food

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Exposure to multiple pesticide residues derived from food is a common occurrence in the general population. Levels of exposure are usually low, below the effective doses. Interactions, such as potentiation and synergism, are not expected to occur at these doses. Available evidence indicates that risk assessment should be carried out for mixtures containing compounds with the same mode of action, since their effects are expected to cumulate. Several methods are available to assess this cumulative risk that differs in the mathematical expression of the data, in the level of uncertainty and transparency, in the requirement of efforts and resources.

Introduction
Exposure to multiple residues of pesticides derived from food is a common event in the general population. In the European Union, 53–64% of the food samples did not contain pesticide residues at detectable levels, 32–42% and 3–5.5% contained residues below and above the maximum residue levels (MRLs), respectively; 14–23% of the samples with residues contained more than one active ingredient (CEC, 2007). Therefore, exposure to mixture of pesticides occurs as a consequence of intake of food items containing different residues or of food items containing multiple residues. Hence, there is scope for conducting the risk assessment of exposure to mixture of pesticides, keeping in mind that doses to which people are exposed are relatively low. This paper will mainly address toxicological issues of exposure to mixtures of pesticide residues in food.

Types of combined action

The toxicological effects of two or more compounds can be described as independent, additive or due to interaction (Wilkinson et al., 2000; Feron & Groten, 2002).

Effects are independent when the mechanism/mode of action (MOA) and possibly, but not necessarily, the nature and sites of toxic effects differ between the chemicals in a mixture, and one chemical does not influence the toxicity of another. The effects of exposure to such a mixture are the combination of the effects that each compound would have caused when given alone (response-addition).

Dose-addition is the effect of exposure to a mixture of compounds that is equivalent to the effect of the sum of the potency-corrected doses of each compound in the mixture. This occurs when all the chemicals in a mixture act in the same way, by the same MOA, and differ only in their potencies. Compounds acting with similar MOA are said to belong to a “common mechanism group” (CMG).

Within the term interaction all forms of joint action that deviate from the two classes of combined toxicity that are described above are included. Deviation can be toward a stronger (synergistic, potentiating, supra-additive) or a weaker (antagonistic, inhibitive, sub-additive, infra-additive) effect than would be expected on the basis of dose-addition for CMG compounds or response-addition for non-CMG compounds.

Relevance to risk assessment for pesticide residues

Exposures to mixtures of pesticides could lead to any of the 3 of those types of combined toxicity. However, low levels of exposure are expected from pesticide residues in food, as indicated by the data in the EU described above. Therefore, it is expected that response-addition will rarely, if ever, occur.

On the other hand, dose-addition and interaction would possibly occur following exposure to mixture of pesticides
as food residues. Dose-addition is expected to cause toxic effects when the sum of the potency-corrected doses is higher than the no observable adverse effect level (NOAEL). In theory, interaction could cause toxic effects even when the exposures to each component present in the mixture in doses are insufficient to cause toxicity. Table 1 summarises the most relevant data of in vivo and in vitro studies with mixtures where doses or concentrations at or below the NOAEL have been included. This table also includes studies with compounds other than pesticides since the issue of mixture is not limited to pesticides. It is worth noting that studies that included such dose levels were a minority of all the studies that addressed the toxicological effects of mixtures, as already underlined (Cassee, Groten, & Feron, 1996; Mason, Borgert, Bus, Mumtaz, Simmons, & Sipes, 2007). From Table 1 it can be concluded that there is evidence that: (a) mixtures of compounds sharing the same MOA show dose-additivity at low doses, (b) at high doses mixtures of compounds sharing the same MOA generally show dose-additivity, although all possible combined effects may occur, (c) mixtures of compounds not sharing the MOA do not show dose-additivity at doses below the NOAEL of each individual compound, (d) mixtures of compounds not sharing the MOA show all possible combined effects (including response-addition, antagonism, synergy) at higher doses.

In conclusion, available data, while not excluding the possibility of interactions leading in some circumstances to toxic effects from combinations of compounds at doses below their individual NOAEL, do support the view that such interactions are less likely to occur at these doses. To this extent, interaction is less relevant to risk assessment for pesticide residues in food than dose-addition of chemicals belonging to a CMG.

How to identify a CMG
Several criteria have been proposed to identify a CMG to be used for a cumulative risk assessment (EFSA, 2007; EPA, 2002; ILSI, 1999). According to EPA (2002), a preliminary identification of substances that might cause a common toxic effect by a common MOA is based on one or more of the following criteria: (a) chemical structure (core molecular structure, functional groups or their metabolic precursors), (b) mechanism of pesticidal action, since it is not uncommon that pesticides are toxic to humans through a mechanism that is similar to that of their activity against their target pests, (c) general mode/mechanism of mammalian toxicity, or (d) a particular toxic effect, since it is conceivable that similar toxic effects might be caused by different compounds via a common MOA. Subsequently, a first refinement of the grouping can be performed by definitively identifying those substances that cause a common toxic effect (i.e. concordant in both site and nature). Then the toxic MOA by which each substance causes a common toxic effect is determined. While desirable, not all of the specific biochemical events leading to toxicity need to be known or completely characterised (i.e. the mechanism of action) but identification of the common key events leading to the toxic effect (i.e. mode of action) is sufficient.

Two considerations can be done on these criteria. On one hand, for the purpose of risk assessment, such a detailed evaluation up to the last step might not be necessary or even possible in all cases. In fact, while consideration of all these criteria will provide the most sound and robust grouping, the lack of data may prevent such refined grouping. Therefore, cumulative assessment has to be performed on such grouping, and refinement will be performed later, if the risk assessment provides unacceptable estimates. On the other hand, there is ample evidence in literature that certain endocrine disruptors show dose-additivity even if they do not share the same primary molecular target (Kortenkamp, 2007 and papers there reviewed). Therefore, the issue is the definition of the concept of common MOA and what this would mean for endocrine disruptors. For instance, compounds affecting the male sexual development via interference with steroid synthesis and not by antagonism of the androgen receptor would not be included in a CMG whereas it has been shown that a mixture of such compounds results in additive effect (Gray et al., 2001; Hotchkiss et al., 2004).
Similar considerations can be applied to estrogenic or estrogen-like chemicals (Picard, 2003). Therefore, it appears that in these cases the criterion for grouping should rather be that of the phenomenological effect (e.g., ano-genital distance for androgens) (Kortenkamp, 2007).

### Methods for cumulative risk assessment of CMG compounds

A number of methods have been proposed for assessing the risk of cumulative exposure to CMG compounds (EPA, 2002; Feron & Groten, 2002; ILSI, 1999; Jonker et al., 2004; Van den Berg et al., 2006; Wilkinson et al., 2000) (see Fig. 1).

<table>
<thead>
<tr>
<th>Type of compounds</th>
<th>Type of study</th>
<th>Effect seen</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPs (same MOA)</td>
<td>In vivo, rats, thermoregulation and AChE inhibition</td>
<td>Additivity</td>
<td>Gordon et al. (2006)</td>
</tr>
<tr>
<td>Nephrotoxins</td>
<td>In vivo, rats, nephrotoxicity</td>
<td>No effect</td>
<td>Jonker et al. (1996)</td>
</tr>
<tr>
<td>Same MOA</td>
<td>In vivo, rats, single and repeated doses, nephrotoxicity</td>
<td>No effect</td>
<td>Jonker, Jones, et al. (1993), Jonker, Woutersen, et al. (1993), and Jonker et al. (1996)</td>
</tr>
<tr>
<td>Different MOA</td>
<td>In vivo, rats, thyroid hormone disruption</td>
<td>Additivity</td>
<td>Crofton et al. (2005)</td>
</tr>
<tr>
<td>PCBs (same MOA)</td>
<td>In vivo, rats, carcinogenicity</td>
<td>Additivity</td>
<td>Walker et al. (2005)</td>
</tr>
<tr>
<td>Dioxins (same MOA)</td>
<td>In vivo, rats, general toxicity</td>
<td>No effect</td>
<td>Jonker et al. (1990)</td>
</tr>
<tr>
<td>Several, unrelated (different MOA and target organ)</td>
<td>In vivo, rats, reproduction and development toxicity</td>
<td>No effect</td>
<td>Heindel et al. (1994, 1995)</td>
</tr>
<tr>
<td>Aldehydes (same MOA)</td>
<td>In vivo, rats, nasal cytotoxicity</td>
<td>No effect</td>
<td>Cassee et al. (1996)</td>
</tr>
<tr>
<td>Several, unrelated (different MOA and target organ)</td>
<td>In vivo, rats, clinical-chemistry and pathology end-points</td>
<td>No effect</td>
<td>Groten et al. (1997)</td>
</tr>
<tr>
<td>Dicarboximides (same MOA)</td>
<td>In vivo, rats, organ weights and sexual hormones</td>
<td>No effect</td>
<td>Nellesmann, Dalgaard, Lam, and Vinggaard (2003)</td>
</tr>
<tr>
<td>Dicarboximides (same MOA)</td>
<td>In vitro, CHO cells, androgen receptor inhibition</td>
<td>No effect</td>
<td>Nellesmann et al. (2003)</td>
</tr>
<tr>
<td>Unrelated pesticides (different MOA)</td>
<td>In vitro, C57BL/6 mouse thyromocytes, apoptosis and necrosis</td>
<td>No effect</td>
<td>Potentiation/synergy</td>
</tr>
<tr>
<td>Metals (similar MOA)</td>
<td>In vitro, human epithelial keratinocytes, cytotoxicity</td>
<td>Additivity</td>
<td>Yang and Dennison (2007)</td>
</tr>
<tr>
<td>Fusarium mycotoxins (same MOA)</td>
<td>In vitro, L-929 mouse fibroblasts, inhibition of DNA synthesis</td>
<td>Additivity</td>
<td>Tajima, Schoen, Feron, and Groten (2002)</td>
</tr>
</tbody>
</table>

In these papers the no-effective dose level was generally 10 times lower than the effective level, most frequently between 3 and 5 times lower. The only exceptions were the experiments by Heindel et al. (1994, 1995) where effective doses were not tested.

*When observed, additivity occurred when the cumulative potency-corrected dose was above the NOAEL.

### Hazard index

The hazard index (HI) is the sum of the ratios between exposure and the reference value (RV) (i.e., acceptable daily intake, ADI, or acute reference dose, ARfD) for each component (hazard quotient, HQ). A ratio of less than 1 means that the estimated/measured exposure is lower than the RV and then the combined risk is considered acceptable. The HI is transparent and understandable, since it relates directly to the RV, which is a long-used and well-understood index of acceptable risk. In addition, since RVs are readily available, application of this methodology is (relatively) rapid and simple, and it can serve as a useful screening method. Refinement of the HI can be performed when the RV of a certain compound is based on an effect that is
HAZARD INDEX

\[ HI = \frac{\text{Exp}_1}{RV_1} + \frac{\text{Exp}_2}{RV_2} + \frac{\text{Exp}_3}{RV_3} + \text{etc} \]

CUMULATIVE RISK INDEX

\[ RI = \frac{\text{RfP}}{\text{Exposure \times UF}} \quad \text{RV} = \frac{1}{\text{HQ}} \]

\[ \text{CRI} = \frac{1}{\frac{\text{Exp}_1}{RV_1} + \frac{\text{Exp}_2}{RV_2} + \frac{\text{Exp}_3}{RV_3}} + \text{etc} \]

REFERENCE POINT INDEX

\[ \text{RfPI} = \frac{\text{Exp}_1}{\text{RfP}_1} + \frac{\text{Exp}_2}{\text{RfP}_2} + \frac{\text{Exp}_3}{\text{RfP}_3} + \text{etc} \]

COMBINED MARGIN OF EXPOSURE

\[ \text{MOE} = \frac{\text{RfP}}{\text{Exposure}} \quad \text{MOET} = \frac{1}{(1/\text{MOE}_1) + (1/\text{MOE}_2) + (1/\text{MOE}_3)} \quad \text{etc.} \]

Fig. 1. Formulae of the different methods for cumulative risk assessment (adapted from Wilkinson et al., 2000).

not the group effect (common toxic effect) or the assessment factor applied includes adjustments not related to the endpoint of concern (adjusted HI).

Cumulative risk index (CRI)

The ratio between the RV and the exposure (i.e. the reciprocal of the HQ) is called risk index (RI). A ratio higher than 1 means that the estimated/measured exposure is lower than the RV. The cumulative risk index (CRI) is the reciprocal of the sum of the HQs. As such, when the CRI is greater than 1 the combined risk is considered acceptable. The CRI is simply a different way of expressing the HI, is not as transparent or understandable as the HI, and involves a more complex calculation.

Reference point index

The reference point index (RfPI) is the sum of the exposures to each compound expressed as a fraction of their respective RfPs for the relevant effect. In this case a common uncertainty factor (UF) is applied to the RfPI and when the result is lower than 1, the combined risk is considered acceptable. As the HI the RfPI is conceptually rather simple and transparent. However, if needed, accommodation for chemical-specific assessment factors should be done earlier in the process and this can be less readily transparent.

Combined margin of exposure

The margin of exposure (MOE) for the individual compound is the ratio of the RfP to the level of exposure in humans (measured or estimated) (i.e. the reciprocal of the ratio used for the RfPI) and then compared to the chosen UF. The combined MOE is called the MOET, and is calculated as the reciprocal of the sum of the reciprocals of the individual MOEs. When the MOET is greater than the chosen UF (usually 100, but an alternative value can be specified by the risk manager) the combined risk is considered acceptable. As for the RfPI, adjustments of individual UF can be done early in the process. Since the MOE is largely used, expressing the results of the assessment as MOET can facilitate communication to the public although the mathematical process is conceptually more difficult to follow.

Relative potency factor (RPF)

This method requires the normalisation of the potency of each compound to a so-called “index compound” (IC) as initially developed for dioxins (in this case called Toxic Equivalency Factor, TEF) (Van den Berg et al., 2006). US EPA used a similar approach for groups of pesticides and this method is also known as relative potency factor (RPF) or potency equivalency factor (PEF) method (EPA, 2002). The potencies can be derived from dose response curves taking the same benchmark dose (BMD) (e.g., the dose causing 10% of a given response) for all compounds or from NOAELs (or LOAELs). Also a combination of BMDs and NOAELs has been used. As an example, if the BMD10 for a compound is 5-fold greater than that of the index compound, the relative potency factor for the compound would be 0.2 and exposures to the compound would be corrected by a factor of 0.2 to convert them to IC equivalents. This is because it would require 5 times more of the compound to produce the same effect as the index compound. The toxicological activity of the mixture is then determined by the sum of the potency-normalised doses and expressed as IC equivalents. This total equivalent exposure is compared to the RV of the IC. If lower than the RV of the IC, the combined risk from exposure to the compounds in the mixture is considered acceptable. An alternative approach would be to determine the MOE of the total equivalent exposure relative to the RfP of the IC. If the MOE is greater than the chosen UF, the risk deriving from the combined exposure is considered acceptable. Additional UF or UFs that are not required for all compounds, such as the inter-species UF for the availability of data in humans, can be applied to the individual RfP (BMD or NOAEL/LOAEL) before calculating the RPF.

It should be noted, also, that all of the methods assume that compounds have parallel dose–response curves, which is not necessarily true and hence is a source of uncertainty. However, since the RfP is usually at the NOAEL/LOAEL the extrapolations and calculations would be performed based on toxicological data that are closer or closest to
the actual human exposures from pesticide residues and therefore having less uncertainty in this respect.

In addition, we should also be aware that NOAELs/LOAELs may be distributed at varying levels along the dose–response relationship curve, depending e.g., on dose-spacing and on the sensitivity of the endpoint used.

Physiologically based pharmacokinetics (PBPK)

This approach is a higher tier assessment that has not been used yet for final risk assessment. PBPK allows the estimation of the concentration of the compound at the target site for a toxicological effect (Teuschler, Rice, Wilkes, Lipscomb, & Power, 2004). There is no expectation that this approach will be consistently used in the near future, in part because they are resource intensive and demanding of specialised expertise.

Cumulative risk assessment of pesticides already performed

Cholinesterase inhibitors

The cholinesterase inhibitors include organophosphates (OPs) and carbamates. Their molecular target is neural acetylcholinesterase (AChE) whose inhibition causes the typical cholinergic syndrome. Each compound has different potency to cause AChE inhibition, and OPs and carbamates differ also because inhibition of AChE by OPs is (almost) irreversible whereas carbamylated AChE reactivates with a half-life of minutes/hours (Karalliedde, Feldman, Henry, & Marrs, 2001). Therefore, repeated exposures to OPs may cause cumulation of effect whereas each exposure to a carbamate that occurs, e.g., every 24 h can be considered as a single acute exposure. Consequently, based on these toxicological characteristics cumulative assessment of OPs should include both acute and repeated exposures whereas that of carbamates includes only acute exposure. The assessment of cumulative acute exposure to both OPs and carbamates would also be considered appropriate. However, different bodies have taken different approaches in this respect. US EPA evaluated separately OPs (repeated exposure only) (EPA, 2006a) and carbamates (1-day exposure only) (EPA, 2007), whereas PSD in the UK (COT, 2002), RIKILT in the Netherlands (Boon & van Klaveren, 2003) and the Danish Food and Veterinary Institute (Jensen, Petersen, & Granby, 2003) evaluated OPs and carbamates together.

US EPA (2006a) selected methamidophos as the IC and toxic potencies for the OPs were determined using brain acetylcholinesterase inhibition from female rats measured at 21 days of exposure or longer; ad hoc experiments were also conducted in EPA. The relative potency was calculated on the central estimate of the BMD10 (benchmark dose causing 10% brain acetylcholinesterase inhibition) using multiple rat studies. Once the RPF had been calculated, UFs, additional to the standard 100, were applied if needed to the RPF of each OP. Finally, estimated potency-corrected exposures were compared to the RfP of the IC and the target MOE was 100 to account for the standard 10× factors for intra- and inter-species differences. Note that this approach is essentially not different from estimating the exposure and comparing it to the IC RV calculated by applying to the BMD10 the standard 100-fold uncertainty factor. In fact, NOAELs/LOAELs for OPs and carbamates used in EU (and elsewhere) to derive the RVs are based on significant 10–20% AChE inhibition. The cumulative MOE was found to be 99–300 in different age groups and as such did not raise concerns about repeated exposures. Even when considering the single day exposures compared to the RIP based on a 21-day exposure, which was a clear overestimate of risk, the MOEs reach the target of 100 for >99.3th percentile for different subpopulations, including children.

For N-methyl carbamates (NMCs), oxamyl was selected as the IC and toxic potencies were determined using brain acetylcholinesterase inhibition in rats after a single exposure (EPA, 2007). The BMD10s for the NMCs ranged over several orders of magnitude. Since for 3 compounds (aldicarb, methomyl and oxamyl) studies in humans were used, the standard inter-species UF of 10×, together with any additional UF if needed, was applied to the other NMCs before calculating the RPF. Thus, only the UF for intra-species differences needed to be taken into account and the target MOE was set at 10 and was reached at 99.848th and 99.870th percentiles of the most exposed age groups (children 1–2 and 3–5 year olds, respectively). The 99.9th percentile MOE was 7.9 and 8.6 for the same age groups, whereas in the other age groups of the population it ranged 12–42.

PSD in UK (COT, 2002) evaluated all OPs and carbamates authorised or found in food during monitoring programmes. The RPF method was adopted either using the NOAEL for erythrocyte cholinesterase inhibition in acute and 90-day rat studies or the ADI/ARfD values, and chlorpyrifos as the IC. In addition, the adjusted HI approach was proposed using the ADI or ARfD but it did not work successfully with the modelling software available at that time. The RPFs defined by PSD were within an order of magnitude of those defined by EPA, most of the difference being due to the application in USA of an additional UF for children as required by USA legislation (FQPA). The two methods of deriving the RPF gave estimated cumulative intakes that differed within a 2-fold factor that had minimal impact on the overall outcome. The exposure assessment was performed using probabilistic software (Monte Carlo risk assessment, MCRA) from RIKILT in the Netherlands. Since all values are expressed as IC equivalents, the application of MCRA in this case is essentially similar to its usual application to an individual compound. It was found that acute exposures on only about 0.1% of consumer days might exceed the ARfD. A number of data limitations have been identified and this preliminary assessment will be completed.
Boon and van Klaveren (2003) of the Dutch RIKILT also used the RPF approach for a preliminary assessment of acute cumulative exposure to OPs and carbamates. They used RfP values from EPA or derived them from data of the literature using the acute NOAELs for either brain or RBC AChE inhibition in any species, including humans. Since they used two ICs (phosphat and acephate) it should be noted that for phosphat the NOAEL was independent of the endpoint, whereas for acephate, the NOAEL for brain AChE was 5 times higher than that for RBC AChE. For this reason the RPFs when using acephate ICs were not consistent when compared to those derived using phosphat. The authors state that when no acute NOAEL was available, it was assumed to be 10 times higher than the NOAEL from repeated dose studies, a clear underestimate of the potency in the case of carbamates due to their toxicological characteristics described above. As expected from the toxicological approach the results of the probabilistic risk assessment of intake were found to depend on the choice of the IC. For instance, the 99.9th percentile of the general population had an estimated intake of anticholinesterases of 268% or 45% of the ARfD of acephate or phosphat, respectively.

The Danish Veterinary and Food Administration assessed cumulative exposure to OPs and carbamates using existing RfPs. The authors considered that the acute exposure estimates were based on such large portions that an individual can consume them only one at a time. Therefore, no cumulative acute risk assessment was deemed useful. Cumulative chronic exposure was assessed deterministically and calculated on the basis of the mean food consumption data and mean pesticide residues data from the Danish monitoring programme. These were well below the ADI even when samples below the limit of quantification (LOQ) were considered to be $0.5 \times$ LOQ. It was also determined that 7 different commodities or 7 of the pesticides accounted for 75–83% or 74–98% of total exposure, respectively (Jensen et al., 2003).

Chloroacetanilides

EPA included in this group acetochlor, alachlor and butachlor because they cause nasal turbinate tumours via a common metabolite that leads to cytotoxicity and regenerative proliferation in the nasal epithelium. The IC was alachlor and the RfP was considered to be the NOAEL for tumour formation. Evaluation of the total risk from exposure to chloroacetanilides in foods indicated that the cumulative MOE was higher than 100 (13,000–53,000) and hence did not raise concern (EPA, 2006b).

Triazines

The ability to cause neuro-endocrine and endocrine-related developmental and reproductive effects was the basis to group 3 of the triazines (atrazine, propazine, simazine) and 3 of their metabolites. Their carcinogenic effects were not considered relevant to humans. All compounds were considered as potent as atrazine. Drinking water was the main source of exposure and the determined MOE was always $>300$. This assessment is being revised (EPA, 2006c).

Comments

The results of the cumulative risk assessment for organophosphates did not give vastly different results, although the analyses cannot be considered fully independent because they used similar data and partly comparable approaches. The EPA cumulative risk assessments for triazines and chloroacetanilides point to estimated exposures that are well below the relevant RV.

In all assessment it was reported that the modelling outputs should not simply be taken at face value, rather it is important to conduct a sensitivity analysis to evaluate the impact of the critical assumptions made during the process.

In essence, the RPF method has in general been used in cumulative risk assessments, but to a different level of refinement (e.g., BMD vs NOAEL). The application of UFs for, e.g., inter-species extrapolation or for age-related differences was considered in different stages of the process, and this may cause some confusion or difficulty in understanding the process. Exposure considerations also led sometimes to the exclusion from the assessment of some compounds.

Conclusions

Exposures to multiple pesticide residues need to be addressed. It is also recognised that monitoring data in EU indicate that exposures to a single pesticide occur at levels that are (well) below the effective dose when the active ingredient is applied according to the regulations. The available data suggest that exposure to a mixture of compounds with different MOAs does not pose an increased risk compared to that of exposure to the individual compounds in the mixture, when exposure occurs below the respective RV, hence below their effective level. Therefore, assessing the risk of exposure to such mixtures is not a priority and efforts should be put on identifying CMGs and to perform the appropriate cumulative risk assessment. However, it might be that a strong biological hypothesis that certain compounds interact below their respective NOAELs warrants closer scrutiny (Groten et al., 2000).

There are several methods to cumulate risk, with different levels of uncertainty and achievable refinement, and a tier approach might be adopted in performing risk cumulative risk assessment.

A number of toxicological and exposure criteria have been identified for deciding on when to perform a cumulative risk assessment (EFSA, 2007). These include groups of compounds that are found in the most consumed food items or with high use based on market analysis, CMG that includes a high number of compounds, groups including compounds with “low” RV — high HQ (e.g., $>0.25–0.5$), evidence from biomonitoring data for the general
population or for sub-populations/geographical areas of “high” intake.

In addition, it should also be noted that presence of relevant non-food sources of exposure might require a cumulative and aggregate assessment to determine what the margin of exposure is for the food part.

Acknowledgements

This paper mostly derives from the work performed for the EFSA Panel on Plant Protection Products and their Residues (PPR). I am indebted to the members of the PPR for discussions and inputs. However, the opinions expressed in this paper should not be considered to be those of the PPR Panel.

References

Boon, P. E., & van Klaveren, J. D. (2003). Cumulative exposure to acetylcholinesterase inhibiting compounds in the Dutch population and young children. Toxicity equivalence approach with acephate and phosmet as index compounds. Wageningen: RIKILT. http://www.rikilt.wur.nl


Legal and illegal colours

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Food additives are evaluated by the European Food Safety Authority’s (EFSA) Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (the AFC Panel). The AFC Panel is supported by its standing working group on food additives (WG ADD), which prepares draft opinions on food additives, including colours, and on the bioavailability and safety of nutrient sources. The WG ADD consists of several members from the AFC Panel together with selected external experts. The draft opinions go forward to the AFC Panel for discussion and final adoption. The adopted opinions are published on the EFSA web site. During its first 5 years of existence the AFC Panel has experienced the highest workload of all EFSA Panels, of which evaluations of food additives have been a substantial part. Although the AFC Panel has issued many opinions on food additives, some of which have been widely debated, such as opinions on aspartame [EFSA. (2006). Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) related to a new long-term carcinogenicity study on aspartame. Opinion expressed on 03/05/2006. Available at http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620765743.htm. Accessed 12.05.08.]

Illegal colours

Following the identification of a number of non-authorised colours in food commodities such as chilli powder, curry powder, and processed products containing chilli powder, sumac, curcuma and palm oil, the AFC Panel was asked to review the toxicology of these dyes illegally present in foods in the EU. The discovery of the illegal dyes triggered wide-ranging withdrawals of contaminated products in some EU countries.

The illegal colours that had been identified in food in the EU were Sudan I, Sudan II, Sudan III, Sudan IV, Para Red, Rhodamine B, and Orange II (see Fig. 1). The Panel performed a literature search to obtain all the available toxicity information on these seven dyes. Following a thorough evaluation of the data the Panel expressed the following opinions (EFSA, 2005).

Sudan I

Sudan I is genotoxic both in vitro, with metabolic activation, and in vivo. Bioassays performed by the US National Toxicology Program (NTP) showed that Sudan I was carcinogenic in the rat but not in the mouse. The genotoxicity and carcinogenicity depend upon the metabolism where fat soluble primary aromatic amines are formed after cleavage of the azo bond (azo-reduction). These primary aromatic amines then can form reactive metabolites.

Sudan II

There were only limited data on in vitro genotoxicity, which however provided sufficient evidence that Sudan II is mutagenic in bacterial tests after metabolic activation. There were also limited and insufficient data on carcinogenicity after ingestion or subcutaneous administration, but the finding of a high incidence of bladder tumours following implantation of Sudan II in the bladder was sufficient to consider this dye possibly carcinogenic.
Sudan III
There were only limited and inconclusive studies available on Sudan III. The structural relationship with Sudan I suggested to the Panel that some identical metabolites might be formed. However, in the absence of data to properly elucidate the distinction between the metabolism of this dye and Sudan I the Panel found it prudent to assume that Sudan III is potentially genotoxic and possibly carcinogenic.

Sudan IV
From the limited mutagenicity data available, the pattern of positive results following metabolic activation was consistent with results obtained with other similar dyes. Thus, the Panel presumed that Sudan IV is potentially genotoxic. There were insufficient data on the carcinogenicity of Sudan IV, but overall the Panel considered that it would be prudent to assume that it is potentially genotoxic and possibly carcinogenic.

Para Red
The data on Para Red were very sparse, but structurally this compound has similarities with other dyes such as Sudan I. In the absence of data the Panel found it prudent to assume that Para Red is potentially genotoxic and possibly carcinogenic.

Rhodamine B
Some studies had shown Rhodamine B to be mutagenic in vitro. However, this might have been due to (unidentified) impurities. The only in vivo data were a positive result in Drosophila melanogaster. The results of three carcinogenicity studies indicated some carcinogenic potential of Rhodamine B. Thus, Rhodamine B was considered to be potentially both genotoxic and carcinogenic.

Orange II
Orange II was not genotoxic in bacterial tests, but a positive effect seen in one in vitro test in mammalian cells and in one in vivo study suggested that activation to a genotoxic metabolite may occur in mammalian systems under certain conditions. The available data on carcinogenicity were considered inadequate for any conclusion.

Overall the Panel concluded on the illegal colours found in food in the EU (EFSA, 2005):

- Data were insufficient for a full risk assessment.
- Sudan I is both genotoxic and carcinogenic and Rhodamine B is potentially both genotoxic and carcinogenic.
- For Sudan II, Sudan III, Sudan IV, and Para Red, conclusive evidence is lacking, but because of structural similarities to Sudan I, it would be prudent to assume that they are potentially genotoxic and possibly carcinogenic.
For Orange II, genotoxicity cannot be ruled out and the existing data on carcinogenicity are inadequate for any conclusion.

Among the azo dyes, the potential to be metabolised to lipid soluble aromatic amines is an alert for genotoxicity and carcinogenicity.

Sulphonation of all ring components, as is the case in most of the azo dyes approved as food colours in the EU, eliminates the genotoxic and carcinogenic activity.

In its opinion, the Panel also offered advice on ways of identifying other dyes, which have structural alerts for genotoxic and carcinogenic properties and which potentially, could appear illegally in food in the EU. The dyes identified were Acid Red 73, Sudan Red 7B, Metanil Yellow, Auramine, Congo Red, Butter Yellow, Solvent Red I, Naphthol Yellow, Malachite Green, Leucomalachite Green, Ponceau 3R, Ponceau MX, and Oil Orange SS (EFSA, 2005).

Re-evaluation of food colours

The European Commission has asked EFSA to re-evaluate all currently permitted food additives in the EU. The re-evaluations of all the food additives will be undertaken by the AFC Panel. The Commission gave highest priority to 46 food colours, which may be legally added to food. The food colours can be subdivided into three groups: natural, synthetic, and mineral colours.

The AFC Panel has outlined a strategy on performing the re-evaluation of the many food additives in a timely manner: A pre-evaluation is performed by the WG ADD to determine whether a full or partial re-evaluation is necessary for a particular additive. This pre-evaluation consists of an information gathering step, which for the food colours has been outsourced to an external contractor (RIVM, Holland), which results in an extensive summary of the available data. The WG ADD, with reference to the AFC Panel, as a second step decides whether a full or partial re-evaluation is needed. Following this decision stage, those additives requiring re-evaluation will be prioritized in the work programme of the Panel.

By November 2007, the WG ADD has considered 14 pre-evaluations and rapporteurs have been appointed among the working group members for the drafting of the opinions to be discussed by the AFC Panel. The 14 colours considered are Red 2G, Allura Red AC, Ponceau 4R, Quinoline Yellow, Sunset Yellow FCF, Tartrazine, Azorubine, Brilliant Blue FCF, Patent Blue V, Brilliant Black PN, Green S, Lycopene, Cochineal Colours and Carmines, and Ammonia Caramel Colour. Draft opinions have been considered by the WG ADD on Red 2G, Allura Red AC, Ponceau 4R, Quinoline Yellow, and Lycopene, and one opinion has been adopted by the AFC Panel (Red 2G; EFSA, 2007).

Re-evaluation of Red 2G

Red 2G is the disodium salt of 8-acetamido-1-hydroxy-2-phenazonaphthalene-3,6-disulphonic acid (see Fig. 2). Red 2G was evaluated by the FAO/WHO Joint Expert Committee on Food Additives (JECFA) in 1981 (JECFA, 1981) and by the EU Scientific Committee for Food (SCF) in 1975 (SCF, 1975). Both committees at that time allocated an Acceptable Daily Intake (ADI) for Red 2G of 0.1 mg/kg body weight based on a long-term rat study with a no-observed adverse effect level (NOAEL) of 8 mg/kg body weight per day for effects on haematological parameters and using a safety factor of 100.

The AFC Panel in its re-evaluation noted that Red 2G is extensively metabolised to aniline by azo-reduction, mainly by bacteria in the gastro-intestinal tract, and that since the previous evaluations performed by the JECFA and the SCF new data had become available showing that aniline was genotoxic in vivo and carcinogenic in rodents. The mechanism behind the carcinogenicity of aniline is not known, there is extensive debate among experts whether the tumours seen in rodents arise due to a genotoxic or a non-genotoxic mechanism. However, the Panel considered that there was insufficient mechanistic evidence to discount a genotoxic mechanism for the rodent carcinogenicity, and based on similar metabolism of aniline in animals and humans a carcinogenic risk for man could therefore not be excluded. Overall, the Panel therefore concluded that it would be prudent to regard Red 2G as being of safety concern since it is extensively metabolised to aniline. Based on these considerations, the Panel withdrew the ADI for Red 2G (EFSA, 2007).

The EU authorities have now removed (EFSA) Red 2G from the list of approved food colours (Commission Regulation (EC) No 884/2007 on emergency measures suspending the use of E 128 Red 2G as food colour).

Food colours and hyperactivity in children

It has been suggested for a number of years that synthetic food colours and other food additives may have behavioural effects resulting in overactive, impulsive and inattentive behaviour in children, referred to as hyperactivity. Children who show this behaviour might to be diagnosed with attention deficit hyperactivity disorder (ADHD).
The Southampton study

In September 2007 McCann et al. in a paper published in the Lancet (the Southampton study) concluded that exposure to two mixtures of four synthetic colours plus a sodium benzoate preservative in the diet result in increased hyperactivity in 3-year old and 8—9-year-old children in the general population. The study had been sponsored by the UK Food Standards Agency. In an earlier study by the same research team there was some evidence for adverse behavioural effects of a mixture of four synthetic colours and sodium benzoate in 3-year-old children on the Isle of Wight (Bateman et al., 2004).

The study consisted of a community based double-blind, placebo-controlled randomised cross-over food challenge in 3-year-old children ($n = 153$) and in 8—9-year-old children ($n = 144$) selected to represent a broad range of behaviour in the general population including children with normal to high level behavioural activity. Children who were medicated for ADHD were not included.

Two mixtures (Mix A and Mix B) each consisting of four different colours and sodium benzoate were given in juice to the children, administered by their parents, on a weekly basis. Mix A contained Tartrazine, Ponceau 4R, Sunset Yellow FCF, Azorubine, and sodium benzoate and Mix B contained Sunset Yellow FCF, Azorubine, Quinoline Yellow, Allura Red AC, and sodium benzoate in the drinks (see Fig. 3).

The duration of the study was 6 weeks. Wash out weeks (Weeks 1, 3 and 5) in which the children received a placebo drink, were alternated with challenge weeks (Weeks 2, 4 and 6) during which the children received either placebo drink, Mix A or Mix B in randomised order.

The behaviour of the children was assessed by their parents in the home, by teachers, and by independent observers in a classroom setting. Behaviour was scored using three standardised and validated hyperactivity behaviour assessment tools. For the 8—9-year-old children behaviour was also assessed via a computer-based attention test.

Ratings of behaviour from each of the individual measures were combined, un-weighted, resulting in an overall weekly Global Hyperactivity Aggregate (GHA) score, representing a novel metric developed by the researchers, combining subjective and objective behavioural measures in one parameter.

Mix A containing Tartrazine (E102), Ponceau 4R (E124), Sunset Yellow FCF (E110), Carmoisine (E122) and sodium benzoate significantly increased GHA scores for all 3-year-old children compared to the placebo control GHA scores (effect size 0.20 [CI: 0.01—0.39], $P < 0.05$).

Mix B containing Sunset Yellow FCF (E110), Carmoisine (E122), Quinoline Yellow (E104), Allura Red AC (E129) and sodium benzoate had no effect on GHA scores in 3-year-old children as compared to the placebo control GHA scores (effect size 0.17 [CI: −0.03 to 0.36]).

![Chemical structures of the food colours and sodium benzoate used in the Southampton study.](image-url)
This result persisted when analysis was restricted to 3-year-old children who consumed more than 85% of juice and had no missing data (complete case group); in this analysis the effect of Mix A in the 3-year-old children was still significantly increased compared to placebo control (effect size 0.32 [CI: 0.05–0.60, \(P < 0.05\)) but for Mix B no significant effect on GHA scores was observed (effect size 0.21 [CI: −0.06 to 0.48]).

For the 8–9-year-old children a significant effect of Mix A (effect size 0.12 [CI: 0.02–0.23, \(P < 0.05\)) or Mix B (effect size 0.17 [CI: 0.07–0.28, \(P < 0.01\)) was seen when analysis was restricted to those children consuming at least 85% of drinks with no missing data (complete case group). When all 8–9-year-old children that completed the study were taken into account, Mix A had no effect on the GHA scores compared to the placebo control (effect size 0.08 [CI: −0.02 to 0.17]) and Mix B had a significant effect on GHA scores (effect size 0.12 [CI: 0.03–0.22], \(P < 0.05\)).

The EFSA evaluation

Shortly following the publication of the Southampton study the European Commission asked the EFSA to assess the results of the study and provide an opinion on the findings. For that purpose, a working group was formed under the AFC Panel, including also outside expertise in ADHD, hyperactivity, allergy, behavioural testing, and statistics. The EFSA also received the original study reports and additional study materials from the UK Food Standards Agency and the researchers.

At the time of this lecture (November 2007) the following points were highlighted for consideration in the EFSA assessment:

- Since mixtures and no individual additives were tested, it is not possible to ascribe the observed effects to any of the individual compounds.
- It was not clear whether the drinks were consumed in the morning before or in the afternoon after school. This may have confounded the outcomes.
- The outcome of parents, teacher, and independent observers scores were combined to give the GHA scores. In this way subjective (parental) scores were combined with more objective scores. Is this appropriate?
- Post hoc analysis revealed that the parental reports were the main contributors to the changes in GHA scores for the 3-year-old children (not to be found in the publication).
- In the 8–9-year-old children the largest contribution to the GHA score came from the computer-based task.
- The researchers suggested that parents may have been more sensitive to, or more exposed to, behavioural changes in their children than the independent observers or teachers. This might have been expected if most of the challenge drinks were consumed at home after school.
- In 8–9-year-old children, when taking all children that completed the study into account, the effects of the two mixtures were opposite to those in the 3-year-olds.

Since then the EFSA AFC Panel has published its opinion in March 2008 (EFSA, 2008). The major conclusions are given in the following extract from the opinion:

The authors’ primary analysis was repeated by the Panel using a more justifiable and conventional statistical model, and this was supplemented by a set of additional analyses. Based on the results obtained it was concluded that the analysis with the recalculated GHA score led to broadly similar conclusions to that in the original paper by McCann et al. (2007), except that the Mix A versus placebo comparison was not statistically significant for the 3-year-olds when all subjects were included (entire sample), while the significance for the ≥85% consumption and complete case groups was increased slightly; and for the 8 to 9-year age group the Mix A versus placebo comparison was no longer statistically significant in any of the three consumption groups.

When the data were analysed without inclusion of the parental scores there were no longer any statistically significant effects of Mix A or Mix B versus placebo, except for Mix B versus placebo in 8-9 year old completers.

Further analyses suggested that the statistically significant effects seen in the 3 year olds (Mix A versus placebo) and in the 8 to 9-year olds (Mix B versus placebo) are largely driven by the parental scores and, in the older males in both comparisons, by the computer score.

The Panel concludes that the McCann et al. study provides limited evidence that the two different mixtures of synthetic colours and sodium benzoate tested had a small and statistically significant effect on activity and attention in some children selected from the general population, although the effects were not observed for all children in all age groups and were not consistent for the two mixtures. The findings may thus be relevant for specific individuals within the population, showing sensitivity to food additives in general or to food colours in particular.

However, it is not possible to assess the overall prevalence of such sensitivity in the general population and reliable data on sensitivity to individual additives are not available.

The clinical significance of the observed effects also remains unclear, since it is not known whether these small alterations in attention and activity would interfere with schoolwork and other intellectual functioning. The clinical significance could possibly be clarified by assessments that used scales for functional impairment and diagnostic interviews, especially if a high proportion
of children with high symptom scores were to be included in such a study.

There are thus a number of uncertainties that are apparent from this new research, some of which are echoed in earlier research. These include:

- the limited consistency of the results with respect to age and gender of the children, the effects of the two mixtures of additives tested and the type of observer (parent, teacher or independent observer);
- the unknown clinical relevance of the novel metric, i.e. the GHA score;
- the unknown relevance of the small effect size;
- the fact that the study has not been designed to identify the effects of individual additives;
- a lack of information on dose-response;
- the lack of a biologically plausible mechanism for induction of behavioural effects from consumption of food additives.

The Panel concludes that the McCann et al. study provides limited evidence that the two different mixtures of synthetic colours and sodium benzoate tested had a small and statistically significant effect on activity and attention in children selected from the general population excluding children medicated for ADHD, although the effects were not statistically significant for the two mixtures in both age groups.

Since mixtures and not individual additives were tested in the study by McCann et al., it is not possible to ascribe the observed effects to any of the individual compounds.

The clinical significance of the observed effects also remains unclear.

In the context of the overall weight of evidence and in view of the considerable uncertainties, such as the lack of consistency and relative weakness of the effect and the absence of information on the clinical significance of the behavioural changes observed, the Panel concludes that the findings of the study cannot be used as a basis for altering the ADI of the respective food colours or sodium benzoate.

Concluding remark

EFSA is continuing its work on the re-evaluation of individual food colours, as part of its longer-term programme to re-evaluate all permitted food additives.

References


GMO risk assessment around the world: Some examples

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All over the world, authorities responsible for the assessment and surveillance of foods and feeds derived using gene technology and the environmental impacts of genetically modified organisms (GMO) have chosen specific strategies to assess their safety. Although different regulatory frameworks are in place, almost all adopted risk assessment strategies are based on a common set of principles and guidelines. Here we provide some examples of these strategies and we compare them to highlight areas where an international consensus exists. Our hope is that even if limited, this short review can represent a first step towards the recognition of an international consensus and a broader dialog on GMOs regulation worldwide.

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0924-2244/\$ - see front matter \textcopyright 2008 Published by Elsevier Ltd.

doi:10.1016/j.tifs.2008.07.007

Introduction

Risk assessment strategies applied in different countries across the world for the assessment and surveillance of foods and feeds derived from organisms modified using modern biotechnology are based on a common set of principles, built on the accumulation of experience and scientific knowledge over the past decades. These principles were first put forward in 1993 (OECD, 1993), and were further detailed by the Ad Hoc Intergovernmental Task Force on Foods Derived from Biotechnology of the Codex Alimentarius Commission (Codex Alimentarius, 2003), an international body jointly established by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) of the United Nations.

During the Scientific Forum organized by the European Food Safety Authority (EFSA) for its fifth anniversary (EFSA, 2007) as a unique occasion to discuss progresses and needs of various issues related to food safety, experts from various part of the world have discussed the experience gained so far in genetically modified organisms (GMO) risk assessment have tried to identify differences and similarities of the risk assessment strategies adopted in some countries, namely Canada, the USA, Australia/New Zealand and the European Union. The focus was primarily on food and feed safety assessment, although attention was also given to some environmental aspects. In the following sections, we try to schematically describe the discussion and the outcome of the Scientific Forum, with the hope to provide a first framework for further understanding of GMO risk assessment around the world.

Foods derived from biotechnology risk assessment

Codex Alimentarius Commission

Codex was created in 1963 by the Food and Agriculture Organization (FAO) and World Health Organization (WHO) to develop food standards, guidelines and related texts such as codes of practice under the Joint FAO/WHO Food Standards Program (www.codexalimentarius.net).

The main purposes of this program are to protect the health of consumers, ensure fair trade practices in food trade, and promote coordination of all food standards work undertaken by international governmental and non-governmental organizations. In 1999, Codex established the Ad Hoc Intergovernmental Task Force on Foods Derived from Biotechnology to develop standards, guidelines or recommendations, as appropriate, for foods derived with

The Principles Document was developed to provide a framework for performing risk analysis on whole foods derived with the use of biotechnology or on components of such foods. While Codex and member countries had had considerable experience performing risk analyses of chemicals intentionally added to or inadvertently present in food (such as food additives, pesticide residues and contaminants), there was, until recently, little experience evaluating the safety of foods themselves.

The Principles Document discusses risk assessment, risk management and risk communication, and describes the safety assessment as a component of the risk assessment. The essence of the safety approach is that the new food (or component thereof) should be compared with an appropriate conventional counterpart, that is, with a food already accepted as safe based on its history of safe use as food. The assessment should follow a structured and integrated approach. It should evaluate both intended and unintended effects, that is, intended and unintended differences from the conventional counterpart; it should identify new or altered hazards; and it should identify any changes in key nutrients that are relevant to human health.

In the Guideline for the conduct of the food safety assessment of foods derived from recombinant-DNA plants the principles for risk analysis of foods derived from modern biotechnology are further detailed. For example, paragraph 4 of the Plant Guideline reiterates that rather than trying to identify every hazard associated with a particular food, a safety assessment should take a comparative approach and identify new or altered hazards relative to the conventional counterpart. Paragraph 5 of the Plant Guideline notes that if a new or altered hazard, a nutritional issue or other food safety concern is identified, one would then need to determine its relevance to human health. If all significant differences are identified and found not to pose safety concerns, then the new food can be considered to be as safe as its conventional counterpart.

The framework for conducting such a safety assessment is outlined in paragraph 18 of the Plant Guideline. It states that the safety assessment of a food derived from a recombinant-DNA plant follows a stepwise process of addressing relevant factors that include:

(A) Description of the recombinant-DNA plant
(B) Description of the host plant and its use as food
(C) Description of the donor organism(s)
(D) Description of the genetic modification(s)
(E) Characterization of the genetic modification(s)

(F) Safety assessment:
   (a) expressed substances (non-nucleic acid substances)
   (b) compositional analyses of key components
   (c) evaluation of metabolites
   (d) food processing
   (e) nutritional modification

(G) Other considerations

The reader can refer to the Plant Guideline itself for further details and pertinent discussion.

GMO risk assessment in the European Union — EFSA initiatives

In the European Union, Member States and EU institutions have agreed a legal framework for the authorization of GMOs. The two main legal instruments for GMO safety assessment are Council Directive 2001/18/EC, which provides the principles regulating the deliberate release into the environment of GMOs, and Regulation (EC) 1829/2003 of the European Parliament and the Council, which strengthens and expands the rules for GMO safety assessment by introducing the ‘one-key-one-door’ approach, namely the need for one authorization to cover both food and feed uses.

Directive 2001/18/EC puts in place a step-by-step approval process made on a case-by-case assessment of the risk to human health and the environment before any GMO can be released into the environment, or placed on the market as, or in, products. The Directive introduces the obligation to propose a monitoring plan in order to trace and identify any direct or indirect, immediate, delayed or unforeseen effect on human health or the environment of GMOs as, or in, products after they have been placed on the market. According to Regulation (EC) 1829/2003, GM food and feed should only be authorized for placing on the market after a scientific assessment of any risk which they may present for human and animal health and, as the case may be, for the environment. The Regulation requires that GM food/feed must not (a) have adverse effects on human health, animal health or the environment; (b) mislead the consumer/user; (c) differ from the food/feed which it is intended to replace to such an extent that its normal consumption would be nutritionally disadvantageous for the consumer/animals.

The European Food Safety Authority (EFSA) has a central role in the independent scientific advice and risk assessment of GMOs, whereas the decision-making with respect to products authorization, inspection and control are the responsibility of the risk managers of the Member States and of the European Commission. The EFSA consults national competent authorities on every GMO application and provides feedback to scientific concerns that are raised by the Member States during the risk assessment process. The EFSA opinions are made available on the EFSA website (http://www.esfa.europa.eu). Subsequently, the European Commission organizes a public consultation before
proposing a draft authorization decision to the mandated Regulatory Committee.

The EFSA Scientific Panel on GMOs has developed guidance documents for the risk assessment of GM plants (EFSA, 2006a) and GM microorganisms (EFSA, 2006b). These guidance documents assist applicants in their preparation and presentation of marketing applications. The GM plant guidance document covers the full risk assessment of GM plants and derived food and feed. The risk assessment process consists of four steps: (i) hazard identification, (ii) hazard characterization, (iii) exposure assessment, which culminates in (iv) an integrative risk characterization. The EFSA guidance, which is regularly updated, follows the specific EU regulatory requirements, and it is based on the comparative assessment approach as developed by the OECD (OECD, 1993) and further elaborated by FAO/WHO (FAO/WHO, 2000) and it is in line with the Codex recommendations (Codex Alimentarius, 2003).

Briefly, the EFSA guidance is based on a two-step logic: (1) identification of possible differences between the GM and non-GM crop, and (2) assessment of the environmental safety, the food/feed safety and the nutritional impact of the identified differences, if any. The guidance defines data requirements and it provides a detailed description of the issues to be considered when carrying out a comprehensive risk characterization. These include molecular characterization of the genetic modification, assessment of the modification with respect to the agronomic characteristics of the GM plant, and evaluation of food/feed safety aspects of the GM plant and/or derived food and feed. Data on composition, toxicity, allergenicity, nutritional value and environmental impact provide, on a case-by-case basis, the cornerstones of the risk assessment process. Key elements for the environmental risk assessment are potential changes in the interactions of the GM plant with the biotic and abiotic environment resulting from the genetic modification. The characterization of risk may give rise to the need for further specific activities including post-market monitoring of the GM food/feed and/or for the environmental monitoring of GM plants.

Recently, the GMO Panel has taken several initiatives to further advance the science of GMO risk assessment and to address specific scientific concerns. An overview of the different initiatives is described below.

The GMO Panel adopted a scientific opinion on the use of antibiotic resistance marker genes in GM plants (EFSA, 2004), which was further complemented with a statement concerning the safety of a specific marker gene: nptII (EFSA, 2007a), where it was concluded that the use of the nptII gene as selectable marker in GM plants does not pose a risk to human or animal health or to the environment.

Under the EU regulatory framework, a new application must be submitted when transgenic traits are stacked (i.e. combined) through the interbreeding of existing GM lines, a strategy which is increasingly being used to combine more and more traits into the so-called ‘multiple stacked events’. Data on the single events are the basis for the risk assessment of stacks, and additional data are required to assess intended or possibly occurring unintended effects which could arise because of possible interaction/s of the stacked genes. The EFSA GMO Panel has developed a specific guidance for the risk assessment of stacked events to address these issues (EFSA, 2007b).

A debated topic in the EU regards the use of animal feeding trials for the risk assessment of GMOs. In particular the value of a subchronic 90-day rodent feeding study on whole food and feed has been the subject of scientific discussion. In 2005, the GMO Panel started considering in depth the potentials and limitations of animal feeding trials for the safety and nutritional testing of whole GM food and feed. This work resulted in an extensive report (EFSA, 2008), where it is concluded that subchronic 90-day rodent feeding study on whole GM plant derived food and feed has sufficient specificity, sensitivity and predictability to act as a sentinel study in order to detect toxicologically relevant differences, as well as nutritional deficiencies/improvements that may be due to the expression of new substances, or alterations in the levels of natural compounds. The report advises that toxicological testing with the whole GM food/feed should be carried out in case the composition of the GM plant is modified substantially, or if there are any indications for the occurrence of unintended effects based on a preceding analysis of the molecular characteristics of the GM organism and/or its agronomic, phenotypic or compositional properties.

Other ongoing work of the GMO Panel includes (1) the consideration of new approaches for the assessment of the potential allergenicity of GM food and feed with particular attention on the use of bioinformatics, in vitro tests and development of animal models; (2) the development of guidance for the risk assessment of GM plants for non-food or non-feed purposes (e.g. molecular farming); and (3) the consideration of strategies for statistical analysis of data generated for the comparative food safety evaluation of GMOs. In particular, the GMO Panel is investigating whether more detailed guidance could be provided to applicants regarding the performance of field trials and statistical analysis of collected data.

EFSA is also giving attention to specific issues of the environmental risk assessment of GM plants which still needs further development such as environmental fitness, effects on non-target organisms, long-term and large-scale environmental effects, broader environmental considerations and the assessment of risk versus environmental benefit. The current case-by-case tiered approach to environmental risk assessment, as outlined in EFSA’s guidance document, is recognized to be very effective; however more specific guidance is needed to assess the potential impact on non-target organisms. The GMO Panel is currently developing more detailed guidance to assess the impact of GM plants on non-target organisms.
Post-market environmental monitoring (PMEM) of GMOs is mandatory in all applications for deliberate release submitted under Directive 2001/18/EC and Regulation (EC) 1829/2003. The PMEM of the GM plant has two aims: (1) to study any possible adverse effects of the GM plant identified in the formal pre-market risk assessment procedure, and (2) to identify the occurrence of adverse effects of the GMO or its use which were not anticipated in the environmental risk assessment. PMEM is composed of case-specific monitoring and general surveillance. The GMO Panel provides guidance for general surveillance of unanticipated adverse effects of the GM plants in theEFSA guidance for the risk assessment of GM plants (EFSA, 2006a).

In summary, the European Union has developed a rigorous and detailed framework for the risk assessment of GMOs, which is in line with internationally agreed procedures. EFSA will continue to further advance the science of risk assessment, update its guidances accordingly, and strengthen co-operation with other national organizations experienced in risk assessment of foods/feeds.

Risk assessment of plants derived from biotechnology:
The US approach

Under US law, food that is adulterated or misbranded may not be introduced or delivered for introduction into interstate commerce (Section 301 of the Federal Food, Drug, and Cosmetic Act — FFDCA http://www.fda.gov/opacom/laws/ffdact/ffdtoc.htm). Adulterated food is defined in part as food that contains any poisonous or deleterious substance that may render it injurious to health, or that contains an unsafe food additive or unsafe pesticide residue (Section 402, FFDCA). An unsafe food additive is one that has not been used according to an authorizing regulation (Section 409, FFDCA). An unsafe pesticide residue is one that has not been granted a tolerance or tolerance exemption (Section 408, FFDCA). The Food and Drug Administration (FDA) has oversight of food additives and the Environmental Protection Agency (EPA) has oversight of pesticides. The implication for biotechnology-derived foods is that if they contain a food additive or pesticide, that food additive or pesticide must have gone through the relevant pre-market authorization procedure by FDA or EPA before the biotech food could be marketed. However, if they do not contain a food additive or a pesticide, they are not subject to any pre-market approval requirement. The fact that a plant was developed using rDNA techniques is not itself a regulatory trigger for food safety oversight or pre-market approval http://www.cfsan.fda.gov/~acrobat/fr920529.pdf.

The FFDCA defines a food additive essentially as a substance whose intended use may reasonably be expected to result in its becoming a component of food or affecting the characteristics of a food, but that is not a pesticide or new animal drug, and is not generally recognized as safe (GRAS) by qualified scientific experts under the conditions of its intended use (Section 201, FFDCA). To date, virtually all new substances introduced into food by biotechnology that are not pesticides have been considered by FDA to be presumptively GRAS, and so have not been subject to food additive approval.

Apart from the presence of food additives or pesticides, foods (biotechnology-derived or otherwise) are still subject to post-market oversight. For example, if a biotechnology-derived food were to contain elevated levels of a native toxicant, such that the food was unsafe, the food would be adulterated and so illegal (Section 402, FFDCA). Or if the composition of the food was changed in a manner such that the food would need to be labeled to indicate that it was different from the usual food with which it would otherwise be confused, the absence of such labeling would render the food misbranded and so illegal. The FFDCA gives FDA broad authority to initiate legal action against a food that is adulterated or misbranded within the meaning of the Act.

FDA has a voluntary consultation process for foods and feeds from new plant varieties, described in a guidance available at http://www.cfsan.fda.gov/~lrd/consulpr.html. The consultation process, although voluntary, has proved to be valuable for developers of food crops using modern biotechnology to resolve questions related to the safety and regulatory status of their foods. As a result, FDA is not aware of any biotechnology-derived food, intended for commercialization in the US market that has not been the subject of a consultation with FDA prior to marketing.

Through the consultation process, developers or firms intending to commercialize a new plant variety for food or animal feed use submit to FDA a safety and nutritional assessment summary containing sufficient information for FDA scientists to understand the approach the firm has followed in identifying and addressing relevant issues. The FDA considers a consultation to be completed when FDA no longer has questions about the firm’s evaluation of the safety and regulatory issues. At that point, it provides a letter to the firm stating that it has no further questions about the food or feed from the new plant variety. FDA publishes on its website the letter and a technical memo describing the information it evaluated (http://www.cfsan.fda.gov/~lrd/biocon.html).

FDA recommends that a consultation ordinarily includes the following information:

1. The name of the bioengineered food and the crop from which it is derived.
2. A description of the various applications or uses of the bioengineered food, including animal feed uses.
3. Information concerning the sources, identities, and functions of introduced genetic material.
4. Information on the purpose or intended technical effect of the modification, and its expected effect on the composition or characteristic properties of the food or feed.
5. Information concerning the identity and function of expression products encoded by the introduced genetic
material, including an estimate of the concentration of any expression product in the bioengineered crop or food derived thereof.

6. Information regarding any known or suspected allergenicity and toxicity of expression products and the basis for concluding that foods containing the expression products can be safely consumed.

7. Information comparing the composition or characteristics of the bioengineered food to that of food derived from the parental variety or other commonly consumed varieties with special emphasis on important nutrients, and toxicants that occur naturally in the food.

8. A discussion of the available information that addresses whether the potential for the bioengineered food to induce an allergic response has been altered by the genetic modification.

9. Any other information relevant to the safety and nutritional assessment of the bioengineered food.

While using somewhat different language, the elements listed above are essentially the same as those recommended for assessment in paragraph 18 of the Codex Guideline. Like Codex, FDA recommends that the new food be compared to an appropriate counterpart, and that intended and unintended changes be identified and their safety determined. New proteins expressed in the food should be assessed for potential toxicity and allergenicity, and the composition of the food should be evaluated, relative to an appropriate comparator, for possible changes in the levels of important nutrients and known toxicants.

As noted above, pesticides introduced into plants by biotechnology are subject to the same FFDCA mandatory pre-market requirements as applicable to conventional pesticides. Such pesticides (including the pesticidal substance, such as a Bacillus thuringiensis delta-endotoxin protein, and the genetic material necessary for its production), are together referred to as “plant incorporated protectants, or PIPs. EPA sets a tolerance level for the pesticide in food (the maximum level at which the pesticide is considered to be safe) or issues an exemption from the requirement of a tolerance for the pesticide because no tolerance level is needed to assure food safety. In the case of PIPs in biotechnology-derived plants, EPA has authorized an exemption from the requirement of a tolerance in all cases to date (http://usbiotechreg.nbii.gov).

As part of its safety review of PIPs, EPA requires direct testing of the pesticidal substance, typically a protein, in an acute oral toxicity study performed on rats or mice. This test is a maximum hazard dose analysis of the protein, intended to uncover any evidence of acute toxicity as observed over a 14 day period. Parameters measured include individual weight gain or loss, behavioral indicators, individual organ weights, any notable pathology upon gross necropsy, and mortality.

In addition to the acute toxicity study, EPA requires assessment of toxicity and allergenicity of the pesticidal protein through comparative database searches for amino acid homologies to known toxins and allergens, in vitro gastric simulation of digestibility and a heat stability examination. The data and information required are consistent with that recommended in the Plant Guideline and allergenicity annex.

Under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA — http://www.epa.gov/oppp00001/regulating/fifra.pdf), plants containing PIPs must receive an EPA registration or experimental use permit before they may be grown in field trials at greater than 10 acres (four hectares) cumulative area. EPA requires information on the plasmid construct used in transformation of the plant, transformation procedures, number of inserts, stability, heritability, DNA sequence, origin of the sequence, expression levels of the pesticidal trait in various plant tissues, and descriptive biology of the source organism and recipient plant (including potential for weediness and invasiveness, and presence or absence of naturally occurring sexually compatible relatives). EPA also requires toxicity assessments for potential environmental impacts, using bird, fish, aquatic invertebrate, insect and estuarine or marine species.

The Animal and Plant Health Inspection Service (APHIS) of the US Department of Agriculture has a complementary role over plants developed using rDNA technology. Under the Plant Protection Act (http://www.aphis.usda.gov/brs/pdf/PlantProtAct2000.pdf), APHIS is responsible for protecting agriculture from pests and diseases. Accordingly, APHIS regulates organisms and products that are known or suspected to be plant pests or to pose a plant pest risk, including those that have been altered or produced through genetic engineering. These are called “regulated articles.” APHIS regulates the import, handling, interstate movement, and release into the environment of regulated organisms that are products of biotechnology, including organisms undergoing confined experimental use or field trials. Regulated articles are reviewed to ensure that, under the proposed conditions of use, they do not present a plant pest risk through ensuring appropriate handling, confinement and disposal.

APHIS regulations provide a petition process for the determination of non-regulated status. If a petition is granted, that organism will no longer be considered a “regulated article” and will no longer be subject to oversight by APHIS. The petitioner must supply information such as the biology of the recipient plant, experimental data and publications, genotypic and phenotypic descriptions of the genetically engineered organism, and field test reports. APHIS evaluates a variety of issues including the potential for plant pest risk; disease and pest susceptibilities; the expression of gene products, new enzymes, or changes to plant metabolism; weediness and impact on sexually compatible plants; agricultural or cultivation practices; effects on non-target organisms; and the potential for gene transfer to other types of organisms. A notice is filed in the US Federal Register and public comments are considered on the environmental
assessment or environmental impact statement and determination written for the decision on granting the petition. APHIS makes available to the public the APHIS environmental review documents as well as a non-confidential copy of the documentation submitted by the person petitioning APHIS for non-regulated status.

In the US, “stacks” (plants containing multiple rDNA traits as a result of conventional breeding among rDNA plants containing different rDNA traits) generally do not receive additional evaluation when the individual traits have successfully completed the FDA, APHIS and EPA procedures. However, plant lines with more than one PIP do need a separate FIFRA registration from EPA for growth on greater than 10 acres, because the combined PIPs would constitute a new pesticide.

Regulation of novel foods in Canada

The globalization of the food supply, the demand for more food sources globally, and the rapid advances in food science and technology have resulted in the introduction of foods not previously available in the marketplace. Novel whole foods and food ingredients may appear through the importation of new products, the introduction of a new species as a food source, the use of new processing techniques, and/or changes in the genetic make-up of the microorganisms, plants and animals from which foods are derived.

In response to these developments, Health Canada promulgated the Novel Foods Regulation under the Canadian Food and Drugs Act on October 27, 1999 (http://www.hc-sc.gc.ca/fn-an/legislation/acts-lois/fdr-rad/division-titre28_e.html). This regulation requires the mandatory pre-market notification of foods intended for sale in the Canadian marketplace that were not previously available or have been modified from their traditional counterpart. Manufacturers and importers are required under these regulations to submit information to Health Canada regarding the product in question so that a determination can be made with respect to its acceptability as food prior to sale. Under the Novel Foods Regulation a “novel food” is defined as follows:

(A) A substance, including a microorganism that does not have a history of safe use as a food
(B) A food that has been manufactured, prepared, preserved or packaged by a process that
(i) has not been previously applied to that food
(ii) causes the food to undergo a major change
(C) A food that is derived from a plant, animal or microorganism that has been genetically modified1 such that
(iii) the plant, animal or microorganism exhibits characteristics that were not previously observed in that plant, animal or microorganism,
(iv) the plant, animal or microorganism no longer exhibits characteristics that were previously observed in that plant, animal or microorganism,
(v) one or more characteristics of the plant, animal or microorganism no longer fall within the anticipated range for that plant, animal or microorganism.

Regulatory oversight for novel foods in Canada is triggered by the new characteristics of the product rather than the process used to create the product. Potential food safety issues are those associated with toxins, contaminants and anti-nutritional factors that could be introduced into the food supply via the importation of new products, the introduction of a new species as a food source, the use of new processing techniques, or changes in the genetic make-up of organisms. To date, Health Canada has authorized the sale of over 100 novel foods (http://www.hc-sc.gc.ca/fn-an/gmf-agm/appro/index_e.html) following a thorough safety assessment of each product. Examples of novel foods approved include: food ingredients such as trehalose and vegetable diacylglycerol oil that did not have a history of safe use; new processes, such as high hydrostatic pressure treated ready-to-eat meats and UV treated apple juice and cider that resulted in a major change in the microbiological safety of these products; and foods derived from genetically modified plants exhibiting new characteristics such as herbicide tolerance and insect resistance.

Given the wide variety of novel foods and the many reasons why a food could be classified as novel, the amount of information necessary for the safety assessment can vary widely from one case to another.

The degree of regulatory oversight necessary for novel food products in Canada is based on the potential risks posed by the product in comparison to its conventional counterpart, where applicable. A risk-based approach is used by Canadian regulatory authorities to protect the consumer while not imposing unnecessary burden on the government and the industry for products that are not truly “novel” food products.

In the case of new plant varieties being proposed for the marketplace, the regulatory trigger is related to the introduction or change in characteristics not previously observed in that plant. Examples include the introduction of new proteins and significant changes in composition.

As mentioned, the characteristics of the new plant variety or final food product derived from that plant determines the need for a pre-market assessment, not the process used to introduce or alter these characteristics. This is based on the fact that many of the issues raised by foods resulting from recombinant-DNA (rDNA) technology (e.g. introduction of new compounds or unintentional compositional changes) are equally applicable to foods produced by conventional breeding techniques such as mutation breeding.

The following two examples of novel rice lines illustrate the product-based approach for evaluating novel foods.

1 “Genetically modify” means to change the heritable traits of a plant, animal or microorganism by means of intentional manipulation.
In the first case, a rice line was developed through chemical mutagenesis, which caused a genetic change that resulted in an alteration to the acetohydroxy acid synthase (AHAS) protein. This mutation allows the plant to grow in the presence of imidazolinone herbicides. In the second case, a rice line was developed using biolistics to introduce the Streptomyces hygroscopicus bar gene and regulatory components necessary for expression. Expression of the bar gene confers tolerance of glufosinate ammonium herbicides. Both of these rice lines were considered a novel food since these rice plants were genetically modified to exhibit new characteristics not previously observed in rice. In both cases, the safety assessment conducted by Canadian regulatory authorities evaluated the new characteristic introduced and the potential for unintended changes in the nutritional and toxic characteristics of the food product.

Canada’s approach to novelty can be viewed as a unique regulatory requirement that can differ from the international standard, especially in the case of non-rDNA or traditionally bred plants. The product-based trigger for regulating new plant varieties in Canada is broader than the process-based system currently used in other jurisdictions due in large part to how genetic modification is defined in the Novel Foods Regulation. However, this approach provides equal regulatory oversight for all developers since any new plant variety, regardless of the genetic modification method used to produce the plant, could pose a risk by the introduction of toxic compounds or changes in the composition of the food product.

In recent years, developers of new plant varieties and other stakeholders have asked Canadian regulatory authorities to clarify the use of novelty as the regulatory trigger. Health Canada’s Food Directorate is moving towards activities to strengthen the risk-based approach to allocating regulatory resources to the pre-market assessment of novel foods to streamline the assessment process for low-risk products. This will involve improving the efficiency of the pre-market assessment process, clarifying novelty triggers, and developing a tiered approach to food risk assessment. Canadian regulatory authorities will continue to seek out opportunities to align the regulatory approach for novel foods with international best practices and harmonize with other nations or international organizations.

Additional information is available at the Health Canada’s novel foods website: www.novelfoods.gc.ca.

GMO environmental risk assessment — The Australian approach

Although there are considerable differences between countries in regulatory structures, environmental priorities (including the preservation of endemic biodiversity) and risk terminology (Hill, 2005), most environmental risk assessments of GMO releases use some form of science-based assessment process that estimates the level of risk through comparison with a non-GM counterpart. In addition, most involve consideration of a range of issues relevant to the overall risk assessment. For GM plants, depending on the introduced trait, these may include toxicity, allergenicity, nutritional profile, agronomic characteristics, increased disease burden, spread and persistence of the GMO, gene flow etc.

This section describes some of Australia’s regulatory experiences with GM plant releases into the environment. Detailed information on the structure of the integrated regulatory framework which involves coordinated decision making by regulatory agencies with complimentary responsibilities, and the assessment processes used by the Gene Technology Regulator (the Regulator) is available in the Risk Analysis Framework (OGTR, 2007).

Under the Gene Technology Act 2000 (http://www.ogtr.gov.au/pubform/legislation.htm), all intentional environmental releases of GMOs, i.e. both field trials and commercial releases, must be licensed. The former are required to be conducted under mandated limits that restrict their size, location and duration, and control measures that are designed to prevent the dissemination and persistence of the GMOs and their introduced genes. The trigger for regulation is the use of gene technology and decisions on whether to issue licenses are based on comprehensive, case-specific, science-based risk analyses. These include consideration of uncertainty in the risk assessment and its potential impact on the risk management measures that might be imposed as license conditions.

Australia has gained the most regulatory experience with GM cotton. The first releases occurred in 1996 under a voluntary oversight system administered by the Genetic Manipulation Advisory Committee. In the seven years since the legislation was introduced in 2001, the Regulator has issued a total of 59 licenses, 50% of which are for GM cotton lines incorporating a diverse range of traits. Of the 10 commercial release licenses, six are for a range of insect resistant and/or herbicide tolerant GM cottons (the others are for GM carnation, GM cholera vaccine, and two GM canola). In addition, Food Standard Australia New Zealand (FSANZ) approved the use of oil and linters from the GM cottons for use in food, and the Australian Pesticides and Veterinary Medicines Authority (APVMA) registered the use of the relevant herbicides on the herbicide tolerant GM cottons and the insecticidal proteins produced by the insect resistant GM cottons. Further information is available from www.ogtr.gov.au/gmorec/ir.htm#table.

After more than 10 years since the commercial release of the first GM cotton line, while some of the original lines have been superseded, more than 90% of the Australian cotton crop is now genetically modified. This has resulted in a number of agronomic changes of significance for future risk assessments of environmental release applications. For instance, the original comparator for GM cotton was non-GM cotton. However, now that the majority is GM, the non-GM parent cotton plant is no longer sufficient as the sole baseline comparator. In addition, the cotton industry has reported a substantial decrease in the use of insecticide...
on cotton crops with associated effects on biodiversity, including increases in the abundance of certain non-target organisms, such as pollinators (e.g. Whitehouse, Wilson, & Fitt, 2005).

Australia has also gained experience in managing the threat of resistance developing to the insecticidal proteins expressed in the GM cotton plants or as a result of transfer of the herbicide tolerance genes. Resistance development is considered to be a product efficacy issue, rather than an environmental risk, and is managed by the APVMA which regulates the use of all agricultural chemicals under the Agricultural and Veterinary Medicines Act 1994. Resistance to insecticides is managed by the APVMA and the cotton industry through conditions placed on the product registration for the use of refugia (to mitigate selection pressure), non-chemical control methods and monitoring for the evolution of resistance. The development of herbicide resistance in weeds is addressed through ‘Best Practice’ guidelines that incorporate integrated management strategies including rotation of herbicides and mechanical weed control methods. These measures are subject to continuous review by an advisory group comprising industry, academic and state government agricultural representatives.

The complexity of environmental risk assessments will increase in the future. While considerations for limited and controlled field trials often focus on the effectiveness of the containment measures, other issues arise where releases with fewer limits and controls are proposed. For instance, the greater the number of GM traits released, the greater the number of novel crosses between these GM plants that will occur (either intentionally or unintentionally). Companies are already projecting releases of more complex, deliberate stacks with around six or more traits combined into a single plant. The possibility of different combinations of GM events as a result of crossing must be included in risk assessments to evaluate the potential human and environmental impacts, and to prevent unauthorized GMOs resulting from these crosses.

In order to maintain appropriate ongoing oversight of commercial releases the Regulator has introduced a mechanism for case-by-case implementation of a program of post-release review (PRR). PRR enables a cautious approach to continue during a release, providing valuable feedback into the risk assessment process and enabling appropriate responses to changing circumstances. License holders are also obliged to advise the Regulator of unexpected or adverse effects. Reports of adverse effects on people or the environment can also be made through a third party reporting system.

Increases in the international trade of GMOs and GM products will also increase the potential for the unintended presence of GMOs in grain shipments that have been approved asynchronously in different jurisdictions. As a result, the Regulator is working with other relevant government agencies and industry to implement a national strategy to manage the unintended presence of unapproved GMOs in imported seeds for sowing.

Consultation is an important component of the operation of Australia’s regulatory system, providing opportunities for a wide range of stakeholders, including the public, to provide input. Ensuring that the risk analysis methodology and processes used are transparent and understandable to all stakeholders enables increased participation and is intended to instill greater confidence in and ownership of regulatory decisions.

Finally, efforts are being made to more effectively incorporate the accumulated knowledge and experience of conventional (non-GM) agriculture and breeding into enhanced risk assessments and the design of effective containment measures for GMOs. A current initiative is to apply nationally accepted standards in weed risk assessment.

The Office of the Gene Technology Regulator participates actively in international efforts that aim to harmonize risk assessments for environmental releases of GMOs and FSANZ is significantly involved in the Codex Taskforce.

Conclusions

A comparison of the mandatory GMO risk assessment strategy implemented by the EU with the voluntary food safety consultation process in the USA, or with the Canadian requirement for a risk assessment of any novel food products shows that a general agreement exists as laid down in Codex (Codex Alimentarius, 2003) where the principles have been developed and accepted. The foundation is the comparative assessment, namely the comparison of the GMO (and/or its derived product/s) with its best conventional counterpart, i.e. a non-GM organism with the closest genetic background to the GMO under assessment, which has gained a history of safe use. The conclusion of this comparison is a risk characterization which should provide an informed scientific guidance for the decision-making process of risk managers.

Experience with environmental risk assessments of GMOs is more limited, and the implementation of international harmonization less advanced than for GMO food/feed risk assessments. Yet, the evaluation of the environmental impact of GM crops relative to the parent plant(s) forms a cornerstone of regulatory decision-making in most jurisdictions and initiatives, ranging from multi-lateral to bilateral, provide important forums for advancement. For example, the Organization for Economic Co-operation and Development (OECD) and the United Nations Environment Program have specific initiatives to provide guidance and to support harmonization across countries. The OECD also publishes resource materials in the form of consensus documents on the biology of different plant species (http://www.oecd.org/document/51/0,3343,en_2649_34385_1889395_1_1_1_1,00.html) for use as a common basis in the conduct of assessments. In the European Union, EFSA organized a dedicated Colloquium to discuss approaches to environmental risk assessment in the light of current scientific thinking and knowledge. Regulatory officials of the US and Canada, recently joined by officials from Mexico,
hold regular technical discussions on environmental reviews of rDNA plants, focusing on molecular genetic characterization and environmental interactions. Canada and the US published two documents describing their approaches to these issues (http://www.inspection.gc.ca/english/plaveg/bio/usda/appenannexe1e.shtml, and http://www.inspection.gc.ca/english/plaveg/bio/usda/appenannexe2e.shtml) and are working with Mexico to update these documents.

Although there is an international consensus recognizing comparative assessment as the core principle for GMO risk assessment, there are still differences in the triggers needed to start the risk assessment itself across countries. In the EU, risk assessment is required for GM foods/feeds and the deliberate release into the environment of organisms which have been genetically modified. Australia also applies this process-driven approach to the evaluation of all applications for intentional release of a GMO, whereas in the US both biotechnology-derived and non-biotechnology-derived products are regulated according to their possible impact on the environment. In Canada the focus is on novelty, i.e. risk assessment is carried out according to the characteristics of a product, regardless of the technology used for its development and production (product-based approach).

Even in the frame of our limited comparison of risk assessment strategies in different countries it is evident the need for further harmonization and standardization of approaches with respect to specific issues such as design of field trials, data requirements including the use of animal experiments, and statistical approaches used for data evaluation. The EU is making efforts in this direction with ad hoc activities (see EU section for further details), but an international consensus is needed to further progress. Harmonization would enhance confidence in the quality and predictability of regulatory processes and benefit trade in food and feed commodities, particularly in view of the globalization of trade and the increasing development and cultivation of GM crops outside the EU.

Our hope is that the limited comparison of GMO risk assessment strategies summarised here can represent a first step towards the recognition of an international consensus. We appreciate that many more steps are needed before harmonization and standardization can be reached, especially with respect to standardization of the assessment of GMO environmental impacts. However, all these efforts will provide a basis for the further development of mutually recognized detailed guidelines for the design and execution of risk assessments of GMOs and derived food and feed products.

Acknowledgments

The authors are very grateful to Dr. Gijs Kletter (RIKILT, Wageningen UR) for his valuable comments and contributions on a previous version of this manuscript.

References


Animal welfare’s impact on the food chain

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The impact of animal welfare on the food chain is considerable. Firstly, an animal’s welfare, its health status, level of stress prior to slaughter etc. has a direct impact on the quality of the product from that animal. The second impact is via citizens, whose strong commitment to animal welfare has led to increasing EU policy in the area. The third is by consumers concerned about the welfare quality of the products they buy. Although linked to food safety, this concern incorporates the whole area of animal welfare including the different criteria inherent in the concept of good animal welfare.

Introduction

Animal welfare is an important attribute of an overall ‘food quality concept’. Recent surveys (‘Eurobarometer’) carried out by the European Commission (European Commission, 2005, 2007a) as well as studies within the EU-funded Welfare Quality\textsuperscript{®} project (Kjaernes, Roe, & Bock, 2007), confirm that animal welfare is an issue of considerable significance for European consumers and that European citizens show a strong commitment to animal welfare. In order to accommodate societal concerns about the welfare quality of animal food products as well as related market demands, e.g. welfare as a constituent aspect of product image, there is a pressing need to develop reliable science-based on-farm systems for assessing the animals’ welfare status (c.f. Blokhuis, Jones, Geers, Miele, & Veissier, 2003).

The scientific study of animal welfare is a relatively young but well established scientific discipline (c.f. Millman, Duncan, Stauffacher, & Stookey, 2004). The area has developed over the last three decades and continues to expand to meet new challenges and new possibilities. It is generally accepted that animal welfare is about the animal itself, and the increasing integration of fundamental biological sciences is contributing towards a greater understanding of the link between the animal’s biology and its welfare state. Parallel to the basic research there is a rapidly growing area of applied animal welfare research directed towards continued improvement of ways to measure the welfare of food producing animals in practice (on-farm, during transport and at slaughter).

At the same time retailers and producers are increasingly recognising that efforts to meet consumer concerns and requirements in the animal welfare area actually represent a business opportunity and may thereby be profitably incorporated in the production strategies of any agri-food company or chain. This also relates to the growing appreciation that conditions negatively affecting animal welfare can also damage other quality aspects. Indeed, improving an animal’s welfare can positively affect numerous aspects of product quality (e.g. reducing the occurrence of tough or watery meat as well as the incidence of bruising, bone breakage, blood spots and abnormal eggshells), and disease resistance (decreasing the immunosuppressive effect of chronic stress and the need for antibiotics); these effects have direct relevance to food quality and safety (Faure, Bessei, & Jones, 2003; Hughes & Curtis, 1997; Jones, 1997, 2001).

Companies are therefore exploring the application of animal friendly husbandry systems, management practices and breeding strategies, the implementation of monitoring and certification schemes, and the communication of the associated information to the consumer (e.g. communication via branding and labelling).

A thorough knowledge of consumer concerns and behaviour is of paramount importance in defining the kind of information that they want about the final products and in developing effective strategies for communicating
welfare standards to the public. The information about welfare obviously also has to be correct and trustworthy, which means grounded in animal welfare science.

Animal welfare science

The number of publications on animal welfare and animal wellbeing is increasing dramatically with over 35,000 ‘hits’ (in Web of Science 13th Nov 2007). Neither is it only in Europe that the research area is developing. Although 46% of these publications could be attributed to authors with an address in Europe, 38% were from North America, 10% Asia, 3% Australia and New Zealand and 3% South America. Such an output reflects the economic importance of the area globally. For simplicity in presenting this short overview of animal welfare science in the context of the food chain, the topic is split into four areas representing the contribution of research related to the animals, their housing, the role of society and animal welfare policy.

The start of animal welfare as a science, particularly farm animal welfare, can be traced back to the 1960’s (Brambell Committee, 1965; Harrison, 1964). Since then there has been an increasing distinction between animal protection (what people do to animals) and animal welfare (the animal’s own experience of its situation) and it is now accepted that animal welfare science is about the animal. The two most widely quoted definitions (Broom, 1996; Duncan, 1993) state that welfare is about an animal’s ability to cope with its environment and, since the concept is only applied to sentient animals, animal welfare is about how animals feel. Thus basic research in this area usually reflects the need to get ‘inside the head’ of the animal.

Dominant fields of research in this area are the behavioural and physiological sciences. For example how the body responds to stress is a key area, with obvious consequences for product quality (Gregory, 1998). It often involves studies of animals’ responses to acute and chronic stress, in particular functioning of the Hypothalamic Pituitary Adrenal (HPA) axis (Mormède et al., 2007), although the concept of allostasis is increasingly being used in the context of animal welfare (Korte, Olivier, & Koolhaas, 2007). But how an animal perceives a situation also affects its responses (Paul, Harding, & Mendl, 2005). Cognition refers to the mental abilities of animals; in particular their perception, reasoning and development of expectations, and is therefore a major determinant in the responses of animals to different situations, not least on the extent to which they are capable of experiencing suffering. Cognition and emotions in animals are therefore important emerging areas in animal welfare science (Boissy et al., 2007; Forkman, Boissy, Meunier-Salain, Canali, & Jones, 2007).

There are of course many additional approaches to those named previously, where the animal and its responses are the focus of attention. It is worthwhile especially mentioning the increasing cross-disciplinary and multidisciplinary approaches that are being used to address these advanced biological questions.

But research in animal welfare science is not only directed at the animal. There is a considerable body of research on housing of animals. Early work focused on comparisons of different housing systems and their effect on animal welfare under controlled conditions. Although useful, such work did not reflect the wide range of housing systems found in practice and research in this area often now takes an epidemiological approach, studying animal health, physiology, behaviour and production under commercial conditions (e.g. Gunnarsson, Keeling, & Svedberg, 1999; Moinard, Mendl, Nicol, & Green, 2003). As a consequence of such studies the importance of stockmanship and management has again come to the fore. For example, in the case of broilers, it has been shown that environment (humidity and temperature in particular) is decisive (under the densities studied) for health and mortality (Dawkins, Donnelly, & Jones, 2004). These environmental factors are determined by management practices and thus a growing trend in animal welfare science is towards developing decision support systems to reduce risk to welfare and identify corrective actions when there is a problem (e.g. Bracke, Hulsegge, Keeling, & Blokhuis, 2004; Jensen & Sorensen, 1998). Decision support systems can also include other aspects of importance to the food chain such as food safety and quality.

Of course society wants to know that animals from which the food they eat is derived did not have poor welfare and, as outlined elsewhere in this paper, ongoing work in projects such as Welfare Quality® are developing methodologies to assess this under commercial conditions. But clearly as much as possible should also be done in terms of prevention. There are many ways in which legislation can be used to protect animals from having reduced animal welfare. There are clear risk factors and it is relevant to identify and control those. This is presented later in the section called ‘Animal welfare and food safety’, so the focus here is on research addressing risk assessment and its relationship to welfare assessment. Many factors of the animal itself (e.g. age and breed), its housing (e.g. type of system and equipment) and in the management (e.g. feeding routines and handling) affect the risk of reduced welfare. Welfare assessment in this way can be considered as measuring the ‘output’, that is to say whether or not these risks were actually realised in a particular case Fig. 1.

While accepting that animal welfare is all about the animal, protecting animals involves some form of input from society, or society as represented by governments, to weigh different aspects (e.g. animal welfare, food safety, economy, environment) and interests of different groups (e.g. consumers, farmers, retailers). How this is best achieved in practice is the final area of animal welfare science to be addressed here. Research in this area addresses the social-economic implications. Although starting to receive attention, there is relatively little work so far other than that carried out on the willingness of consumers to pay for products produced under welfare friendly conditions.
pared to birds without (Gross & Siegel, 1979). Productive human contact had better food conversion ratios compared to welfare assessment (right-hand side of the diagram) where the current welfare status of the animal is measured. The welfare of an animal is the outcome of the various housing, management and animal risks. 

(e.g. European Commission, 2005, 2007a; Napolitano, Pacelli, Girolami, & Braghieri, 2008). But increasingly research is being directed towards the willingness of farmers to change to more welfare friendly practices and how this varies according to the cost of implementing these changes.

On the other hand, there is ample evidence from animal science that various welfare improvements will help to reduce costs or provide other benefits to farmers. Providing pigs with more space will improve growth rate (e.g. Edwards, Armsby, & Spechter, 1988; Gonyou et al., 2005). Commercial broiler chickens which received frequent positive human contact had better food conversion ratios compared to birds without (Gross & Siegel, 1979). Productivity of dairy cows is markedly influenced by the fear of people (gently versus aversive handling during milking) (Rushen, DePassille, & Munksgaard, 1999).

Ultimately the increased understanding of which indicators reliably reflect an individual animal’s welfare status and what factors represent a risk to good welfare, needs to be implemented in practice. Indeed there is considerable pressure on animal scientists to deliver valid, reliable and at the same time feasible measures for such implementation. A major contribution of animal welfare science in the context of the food chain therefore is to provide the basis for practical welfare assessment and to assist in the process whereby this animal welfare science knowledge is combined with our knowledge of food science for inclusion in European Union (EU) policy.

**Implementation of animal welfare assessment in practice**

As indicated above, there is a pressing need for credible on-farm assessment systems to determine the animals’ welfare status. These systems should provide a standard way of converting science-based welfare-related measures into information that is conveyable to and easily understood by the consumer, thereby addressing specific concerns and allowing for the clear marketing and positioning of the product.

The Welfare Quality® (WQ) project aims to deliver such on-farm welfare assessment systems for several species as well as accepted procedures for the standardised conversion of welfare measures into accessible and understandable product information. The focus within the WQ project is to measure parameters at the animal level that reflect the actual welfare state of the animals. Such animal-based measures (or ‘output measures’) include the effects of variations in farm management systems as well as the effects of specific system—animal interactions. A set of design measures (i.e. characteristics of the housing system such as floor type, size of pen, ventilation, etc.) and management measures (handling, environmental enrichment etc) will also be included so that the causes and risks of poor welfare can be identified and appropriate remedial measures proposed.

Obviously, the set of measures to assess welfare must address all important welfare criteria. In WQ twelve welfare criteria, grouped under four principles, were identified (see Table 1) (Keeling & Veissier, 2005; Veissier & Evans, 2007).

To investigate if the above list of criteria reflects the concerns of citizens and if they consider these relevant for assessing the ‘animal friendliness’ of the products available on the market, focus groups interviews were carried out in seven study countries (Italy, France, Hungary, UK, the Netherlands, Norway and Sweden) (Miele & Evans, 2005). The results showed that the participants in the focus group discussions reacted favourably to the ‘experts’ list of areas of concern as reflected in the criteria in Table 1. Most participants identified more commonalities than differences between their understanding and the scientific approach to what is important in defining the welfare of animals.

Procedures are also being developed for an overall assessment of animal welfare to allow different farms and slaughterhouses to be awarded different levels or grades according to the welfare of the animals in their unit. Such an overall rating should help to convey the results of welfare assessments to consumers in a transparent and credible way. This process is illustrated in Fig. 2.

There are obviously various ways of implementing assessment systems and of conveying generated information to consumers. Companies may for instance implement such systems to brand their product or existing quality product labels may integrate this welfare ‘module’. It could also be implemented through an EU regulation on voluntary labelling, in line with the currently existing regulations on labelling of organic products (EU Council Regulations, 2092/91; 1804/99) or PDO/PGI (typical products) (EU Council Regulation 2081/92).

Clearly, the ultimate use and implementation of the assessment and information systems are dependent upon many different actors, trends and opportunities, such as the producer, breeding, retail and food service industries, citizen’s engagement, NGOs activities, political developments at EU or global levels, and market developments. Indeed, it is very likely that a major effort by market actors will be required to realise the uptake of these systems and strategies in their product chains and markets.
Animal welfare and food safety

The European Food Safety Authority (EFSA) provides independent scientific advice regarding risks associated with food and feed, plant health, environment, animal health and animal welfare. Since 2003, the Animal Health and Welfare (AHAW) Panel of EFSA has adopted 14 Scientific Opinions on Animal Welfare and eight of those also addressed the impact on food safety (see Table 2). EFSA is currently working on opinions on the welfare of dairy cows and welfare of different fish species and also in those opinions the food safety implications are considered.

Since 2004, the possible implications for food safety involving other areas of expertise in EFSA, like Biohazards, Contaminants, and Plant Health, have been considered in most scientific opinions on animal welfare, thus illustrating the possible synergies or divergences between animal welfare, animal disease and food safety. For instance, giving chickens access to open air may improve their welfare but, on the other hand, it may increase the risk of exposure to contagious diseases such as Avian Influenza (EFSA, 2006c). In the scientific opinion on the welfare of laying hens it was concluded that the risk of contamination with Salmonella spp. and particularly Salmonella enteritidis might be higher when eggs are produced in some non-cage systems because of the greater exposure of layers and their eggs to environmental contamination (EFSA, 2004a). Similarly, different floor types and space allowances in pig farming and husbandry might have a role in the spread of infectious agents between farmed pigs (EFSA, 2004b). Space availability per pig, social interactions and total number of animals in the group may influence the dispersal of pathogen spread by the oro-faecal or respiratory routes. Bedding material (straw) might act as vehicle of diseases such as Foot and Mouth Disease (FMD) or Aujeszky’s disease. Floor types that cause abrasions to feet or snout are likely to facilitate the spread of vesicular diseases such as FMD or Vesicular Stomatitis. When pigs are kept outdoors, contact with wild fauna, mainly wild boars, is believed to be a source of Brucellosis in domestic pigs. The main food safety concern regarding some methods of animal stunning and killing is related to “penetrating captive bolt” stunning of ruminants and the potential dissemination of central nervous system emboli into edible tissues with consequent exposure of humans to transmissible spongiform encephalopathies (TSE) (EFSA, 2005; EFSA, 2007b). Transportation of animals, as well as mixing animals in the staging points, can also be an efficient mode of spreading infectious animal diseases such as FMD (EFSA, 2003).

Whenever possible, EFSA uses a Risk Assessment (RA) approach as a basis for its opinions. The RA methodology applied to animal welfare can support the scientific prioritization of welfare issues for the further implementation of animal welfare standards. While specific RA guidelines have been published on animal diseases or chemical substances by the World Organisation for Animal Health (OIE) (OIE, 2004a, 2004b) and World Health Organisation (WHO) (1999) respectively, no specific international guidelines on RA for animal welfare are currently available (EFSA, 2006a).

Table 1. Welfare principles and criteria identified in welfare quality® (adapted from Veissier & Evans, 2007)

<table>
<thead>
<tr>
<th>Welfare principles</th>
<th>Welfare criteria</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good feeding</td>
<td>1. Absence of prolonged hunger</td>
<td>Animals should not suffer from prolonged hunger</td>
</tr>
<tr>
<td></td>
<td>2. Absence of prolonged thirst</td>
<td>Animals should not suffer from prolonged thirst</td>
</tr>
<tr>
<td>Good housing</td>
<td>3. Comfort around resting</td>
<td>Animals should be comfortable, especially within their lying areas</td>
</tr>
<tr>
<td></td>
<td>4. Thermal comfort</td>
<td>Animals should be in a good thermal environment</td>
</tr>
<tr>
<td></td>
<td>5. Ease of movement</td>
<td>Animals should be able to move around freely</td>
</tr>
<tr>
<td>Good health</td>
<td>6. Absence of injuries</td>
<td>Animals should not be physically injured</td>
</tr>
<tr>
<td></td>
<td>7. Absence of disease</td>
<td>Animals should be free of disease</td>
</tr>
<tr>
<td></td>
<td>8. Absence of pain induced by management procedures</td>
<td>Animals should not suffer from pain induced by inappropriate management</td>
</tr>
<tr>
<td>Appropriate behaviour</td>
<td>9. Expression of social behaviours</td>
<td>Animals should be allowed to express natural, non-harmful, social behaviour</td>
</tr>
<tr>
<td></td>
<td>10. Expression of other behaviours</td>
<td>Animals should have the possibility of expressing other intuitively desirable natural behaviours, such as exploration and play</td>
</tr>
<tr>
<td></td>
<td>11. Good human–animal relationship</td>
<td>Good human–animal relationships are beneficial to the welfare of animals</td>
</tr>
<tr>
<td></td>
<td>12. Absence of general fear</td>
<td>Animals should not experience negative emotions such as fear, distress, frustration or apathy</td>
</tr>
</tbody>
</table>

Fig. 2. Welfare assessment is preferably based on measures at the animal itself to reflect the quality of housing and management as experienced by the animal itself. In the Welfare Quality® project a measuring system is developed to assess compliance with 12 criteria that cover the different domains of welfare. To facilitate communication to consumers, these criteria are combined in four principles and finally in an overall assessment of animal welfare.
Table 2. Examples of interaction between animal welfare, animal disease and food safety with possible impacts on public health from the EFSA Scientific Opinions.

<table>
<thead>
<tr>
<th>Animal welfare issue</th>
<th>Impact on animal disease</th>
<th>Impact on food safety</th>
<th>Impact on public health</th>
<th>EFSA scientific opinion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outdoor systems for poultry</td>
<td>Exposure to Avian influenza by migratory birds</td>
<td>Egg contamination with <em>Salmonella</em> (S. enteritidis)</td>
<td>Avian influenza in humans HPAI Salmonella in humans</td>
<td>EFSA, 2004c</td>
</tr>
<tr>
<td>Non-cage systems in laying hens</td>
<td>Risk of egg contamination with <em>Salmonella</em></td>
<td>Only indirect effect on the increase of pharmaceutical administration.</td>
<td>Only indirect effect on presence of antibiotics in meat and resistance development.</td>
<td>EFSA, 2004b</td>
</tr>
<tr>
<td>Low space allowance</td>
<td>Spread of infectious agents e.g. FMD, PRV, CSF, SVD, ASF. Especially in growing-finishers pigs: post-weaning enteric disorders, swine dysentery, PMWS, swine influenza, PRRS, pneumonia.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bedding material e.g. straw</td>
<td>Vehicle of FMD or Aujeszky disease.</td>
<td>Indirect effect only.</td>
<td>Indirect effect only.</td>
<td>EFSA, 2004b</td>
</tr>
<tr>
<td>Floor types e.g. floors causing abrasion to feet or snout</td>
<td>Spread of FMD, Vesicular stomatitis.</td>
<td>Indirect effect only.</td>
<td>Indirect effect only.</td>
<td>EFSA, 2004b</td>
</tr>
<tr>
<td>Pigs kept outdoor in contact con wild fauna</td>
<td>Brucellosis contamination.</td>
<td>Indirect effect only.</td>
<td>Indirect effect only.</td>
<td>EFSA, 2004b</td>
</tr>
<tr>
<td>Stunning methods e.g. penetrative captive bolt in ruminants</td>
<td>No</td>
<td>Dissemination of contaminated nervous tissue into edible tissues.</td>
<td>BSE/CJD risks.</td>
<td>EFSA, 2005; EFSA, 2007b</td>
</tr>
<tr>
<td>Mixing animals in staging points</td>
<td>Spread infectious diseases among animals e.g. <em>Escherichia coli</em>, <em>Salmonella</em>, and highly contagious animal diseases (e.g. FMD).</td>
<td>Meat contamination with <em>Salmonella</em>.</td>
<td><em>Salmonella</em> in humans.</td>
<td>EFSA, 2003</td>
</tr>
</tbody>
</table>

In 2006, a first approach towards RA for animal welfare was developed in connection to scientific opinions on the welfare of calves (EFSA, 2006b) and the import of captive birds (EFSA, 2006c). Subsequently, the recent scientific opinions on Pig Welfare (EFSA, 2007a) and Fish Welfare (EFSA, 2008) also followed a similar RA approach. A clear description of the causal factors (hazards) and the quantification of the adverse effects on animal welfare, which are crucial steps for the risk characterization, were the main difficulties encountered (Candiani et al., 2007).

Several categories of hazards should be evaluated for assessing the risks in animal welfare, such as species, age, production system, environmental conditions, food and feeding, husbandry and management, genetics, physiological status, diseases and impact of control measures, and some of them have possible direct or indirect impact on food safety (Müller-Graf et al., 2008). As explained earlier, these risks in turn affect the output measures used in welfare assessment and listed in Table 1 i.e. those indicating prolonged hunger, absence of disease, good human—animal relationship and so on.

EFSA launched in September 2007 a self-mandate (EFSA-Q-2007-168) for the development of RA Guidelines and working methodologies for animal welfare. This will include a harmonized definition of animal welfare, the classification of the main animal welfare issues and animal categories to be considered and, for each animal welfare issue, the risk factors (hazards) and the consequences (adverse effects) potentially affecting the animals. A comprehensive list of welfare indicators for each species and each welfare issue will be provided and different scenarios, depending on the species and production systems, will be considered. The animal welfare issues to be considered in these guidelines have been divided in four main categories: i) stunning and killing, ii) transport, iii) housing and iv) management.

The RA methodology applied to animal welfare, currently under development, will consider the impact of all mentioned aspects in a global and integrated way, not only focusing on welfare aspects (including animal health), but also considering factors related to food safety. The outcomes of the methodology and the identification of welfare indicators may allow the establishment of control and monitoring plans at farm level, ensuring a high quality farm to fork food chain.

**EU policy on animal welfare**

In the European Union (EU), legislation on animal welfare has a longstanding tradition in many Member States, going back to the 19th century. Looking back, the first European Community legislation on animal welfare was adopted in 1974: Council Directive 74/577/EEC of 18 November 1974 on stunning of animals before slaughter. The discussions around this Directive indicate the importance that was then already attached to animal welfare and in particular to the prevention of unnecessary suffering. Following this Directive, the Protocol on Protection and
Welfare of Animals annexed to the EC Treaty in 1999 (the Treaty of Amsterdam amending the Treaty on European Union) represents a milestone for the development of the Community’s animal welfare policy, highlighting the ethical dimension of this policy. This Protocol spells out the obligation to pay full regard to the welfare of animals as sentient beings when formulating and implementing the Community’s policies. The legal recognition of animals as “sentient beings” has been recently reconfirmed by the Lisbon Treaty in 2007.

The link between animal welfare, animal health and food safety was subsequently highlighted in 1999 in the Community White Paper on Food Safety (European Commission, 2000), integrating animal welfare into the food chain policy of the EU as well as in the recent new Animal Health Strategy 2007–2013 (European Commission, 2007b). Furthermore, recent reforms of the EU’s Common Agricultural Policy strengthened the role of animal welfare considerations by introducing the ‘cross-compliance’ principle — making compliance with animal welfare requirements a condition of certain farm subsidies.

In this context and while globalisation is revolutionising international trade in live animals and animal products, animals are more and more at the centre of ethical concerns modulated by cultural and traditional values. In particular, today the farming of animals is no longer viewed by European consumers simply as a means of food production. Instead, it is seen as fundamental to other key social goals such as food safety and quality, safeguarding environmental protection, sustainability, and enhancing the quality of life in rural areas (Gavinelli, Rhein, & Ferrara, 2007).

There is a considerable interest in animal welfare standards (European Commission, 2005, 2007a), which is reflected in the demand for more information and in the awareness that European public commitment is relevant for higher welfare standards. Animal welfare is an issue that Europeans rank highly, giving it 8 out of 10, on average, in terms of importance. Sixty-two per cent of EU citizens would change their shopping habits in order to access more animal welfare friendly goods, which are seen as being healthier and of higher quality.

Globalisation of trade in animal produce in association with increasing demand for animal protein has resulted in a sharp increase in the number of animals slaughtered for meat worldwide. In the case of species such as pigs and chickens, which are normally kept in the most intensive forms of housing, the establishment of industrial farming plants in developing countries often competes with local farming activities. This raises several concerns about the sustainability of this form of production, in particular their impact on the environment and the countries’ social structures (World Society for the Protection of Animals, 2007). As reported by the Food and Agriculture Organisation of the United Nations (FAO, 2006), livestock play an important role in the current environmental crisis. They can lead to biodiversity loss and ecosystem changes as a result of habitat change, climate change, overexploitation and pollution, at the local and global level.

Concern about the impact of food production on the life of rural populations has led to increased awareness of the need to develop animal farming systems able to interface the production process with the social and cultural aspects of the region where they are located and with the specific product characteristics. This is most evident in certain regions of Europe where local examples of food labels that identify high quality products both in terms of their traditional heritage and the ethical and cultural implications are found.

In this context, the overall aim of the European Commission’s initiative is to initiate a broad public debate on animal welfare which will allow shaping a coherent, comprehensive and widely accepted policy in this field both in the EU and with its major trading partners. In this regard the purpose of the Community Action Plan on the Protection and Welfare of Animals 2006—2010 (European Commission, 2006) is to ensure that animal welfare is addressed, in all EU sectors and through EU relations with third countries, in related policy fields such as agriculture, environmental policies and research taking into account the socio-economic impact of any new measures. This coordinated approach to animal protection and welfare should be complemented by initiatives to improve consumer information and awareness. Since informed consumers can support, with their purchasing behaviour, the development of sustainable farming practices, the Community Action Plan suggests introducing improved marketing, labelling and communication strategies in order to meet this goal.

An animal welfare labelling clearly identifying the ‘level’ of welfare applied is essential, on the one hand, to enable consumers to make an informed choice and, on the other hand, to enable producers to benefit from market opportunities. Such a system of classification needs to be based on standardised scientific indicators well recognised both in the EU and internationally, and underpinned by research, in order to facilitate the marketing of these products (Gavinelli et al., 2007). The application of such a system of classification of welfare standards is relevant and well received also in the ongoing global debate on the issue. In this regard, the Commission continues to support and to initiate international initiatives on animal welfare to create greater consensus and raise awareness on animal welfare (European Commission, 2002, 2006) through also the promotion of scientific partnership with countries trading with the EU. In particular, the European Commission is committed to work with international organisations such as the World Organisation for Animal Health (OIE) for promoting the development and implementation of internationally recognised animal welfare standards.

The Community Action Plan on the Protection and Welfare of Animals 2006—2010 states that the EU should engage and assist developing countries in implementing animal welfare measures, in order to create a win—win-situation.
for all actors. The Community approach is in line with the International Finance Corporation statement that ‘good standards of animal welfare are considered as a prerequisite for sustainable agricultural production which create opportunities for trade worldwide’ (International Finance Corporation-World Bank Group, 2006a, 2006b).

The Community is building on these assumptions in particular supporting the vision of integrating the farming of animals in good health and welfare conditions with the respect of several other issues such as the safety of the products and the respect for the environment: this integrated approach will bring a real benefit for the global society and will be able to ensure a positive future trend for the global trade of animal products and for agriculture.

Concluding remarks

Farm animal welfare is of increasing significance to European citizens and consumers and it is becoming increasingly recognised as an important attribute of food quality. This constitutes an important impact on the food chain as it requires visibility of production processes and an understanding of how they affect welfare as well as transparent systems to guarantee welfare quality of animal-based food.

Welfare researchers are providing the scientific basis for reliable and feasible welfare assessment systems and standardised tools for the conversion of welfare measures into accessible and understandable information, thereby addressing concerns and allowing clear marketing and profiling of products.

EU legislation based on scientific evidence and systematic risk assessment by EFSA is important to support the further improvement of animal welfare in Europe.

Considerations about animal welfare should also take account of the potential risks related to food safety, animal health and the spread of animal diseases. The critical relationship between animal health and animal welfare is internationally recognised and, since 2003, has been a basic principle inspiring the activities of the World Organisation for Animal Health (OIE). This perspective is now leading legislators and risk managers to change their approach to the management of these issues and, where possible, integrate animal health, animal welfare and food safety into a unique legal framework. However, interactions between the three concepts; animal welfare, animal disease and food safety need to be further explored scientifically and any potential impacts in the food chain better quantified in order to make the full system more efficient and more adaptable to new scientific evidence as it becomes available.

Acknowledgements

The authors like to acknowledge the valuable contributions to this paper by Maria Ferrara, Denise Candiani and Oriol Ribó.


Animal cloning for food: epigenetics, health, welfare and food safety aspects

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Cloning via somatic cell nucleus transfer (SCNT) is a potential way for using validated genomes in farm animal breeding and to save endangered breeds or species. Although this technique is relatively inefficient and costly, it is envisaged to use it as an assisted reproduction technique. Despite numerous problems observed in the perinatal period, after some time clones appear normal although they may have kept some epigenetic modifications. Meat and milk from cattle and meat from pig clones and their offspring are substantially equivalent to conventional animals with no observed toxicity or allergenicity. Due to limited data, monitoring of clones and their offspring is recommended to detect whether there are unexpected long-term effects of cloning.

Introduction

Cloning is a way to control genetic selection by preventing the random distribution of parent genes into offspring that takes place in sexual reproduction and makes possible the use of genomes from validated genitors. Cloning can be used in breeding to accelerate the introduction of desired traits into herds. Cloning via SCNT according to the technique used to generate “Dolly the sheep” is laborious and relatively costly but it has been sufficiently improved to make possible its implementation to improve genetic selection of farm animals and particularly of cattle and pigs. Interestingly, cloning can be applied similarly to both sexes making it possible a more intensive use of specific female genotypes with desired phenotypes compared to conventional breeding.

The cloning technique implies a reprogramming of the genetic elements of a somatic cell to transform it into a totipotent embryonic cell. This process is complex and sometimes not fully complete in all instances. The incomplete dysfunctional reprogramming is responsible for the morbidity and mortality of clones during pregnancy and soon after birth. After the juvenile period (up to 6–12 months), cattle clones appear normal although they could keep some epigenetic modifications which seem not to be transmitted to progeny.

Clones are precious genitors primarily intended to be used for breeding and not as human food. On the contrary, offspring of clones are valuable candidates to enter the food chain. The cloning technique in its present state induces a significant reduction of animal health and welfare in a cohort of animals which must be balanced with the advantages cloning may bring for breeders and consumers but also for the animals themselves (e.g. using a cell donor with proven resistance to infection). Cattle and pig clones and their offspring are the two species first likely to be used for human food.

Cloning technique

SCNT is a complex technological procedure, with many steps potentially affecting the reprogramming process, creating different epigenetic patterns and aberrations and thus influencing the foetal development and long-term health of the clone. Due to these processes, the animal health and welfare are influenced by the sum of the effects of the technical steps which consequently may affect the food safety of products derived from the clones. Many of the technology improvements are based on empirical scientific studies, as the exact nature of the interactions is often unclear. Existing information demonstrated major influence by some of the technical components, including the activation methods used, in vitro culture conditions and treatment of donor cells or reconstructed embryos with chemicals affecting the methylation processes.

There are numerous variations in the technical steps and applied parameters in SCNT, although all cloning methods
are fundamentally similar by achieving the replacement of the oocyte’s original nucleus with that from a somatic cell. Three protocols can be distinguished based on the presence or absence of the zona pellucida (a coat surrounding the oocyte) during enucleation and reconstruction of the new model embryos: (1) “conventional” zona-intact SCNT, (2) zona-free SCNT and (3) zona-free “hand-made cloning". The first method has been used for a longer time, and is still the most popular. It can be used to enucleate oocytes in the metaphase II (MII)-arrested developmental stage. In this method the genetic material from the recipient oocyte is removed by aspirating the maternal chromosomes envelopes in order to obtain a so-called cytoplast. This SCNT procedure is very labour-, skill- and technology-intensive due to complicated micromanipulation steps (Cibelli et al., 1998). The second method is simpler, using zona pelluccida-free oocytes which can increase the embryo clone and offspring throughput production and is also easier to establish in a production environment (Oback et al., 2003). The third method is using manual sectioning of zona-free oocytes with a microblade and then the cathe- tin-free portion is fused with another cytoplast to restore the original volume before SCNT (Vajta, Lewis, Hyttel, Thouas, & Trounson, 2001). The different SCNT protocols applied by different users confound the understanding for the biological causes underlying the low efficiency of SCNT and it is practically impossible to standardize all experimental parameters with inherited differences in operator skills, oocyte quality and donor cells (Gao et al., 2004).

The core elements of improving the SCNT procedure are the following: choice of donor cells, the recipient cytoplast, and certain modifiers of the reprogramming. Perfecting donor cell culture methods and using low passage number of such cells could help to decrease epigenetic modifications, genetic aberrations and mutations which can accumulate during cell culture (Niemann, Tian, King, & Lee, 2008). The genetic origin of the cells is an important factor according to data mostly generated in mouse. The cell cycle status of the donor cells plays an important, but still controversial role, and several studies reported on the positive effect of serum starvation induced GO (Wilmut, Schnieke, McWhir, Kind, & Campbell, 1997) or cell culture confluency induced G1 stage (Cibelli et al., 1998; Li, Li, Jouneau, Zhou, & Renard, 2003).

Cytoplasts for SCNT are often generated by enucleation of non-activated MII stage oocytes (Cibelli et al., 1998; Wilmut et al., 2002; Wilmut et al., 1997), but Telophase II stage oocytes (Bordignon & Smith, 1998) and mitotic zygotes are also viable options (Greda, Karasiewicz, & Modlinski, 2006; Schurmann, Wells, & Oback, 2006). In vitro approaches to increase SCNT success include treating donor cells with pharmacological agents to modify their epigenetic marks (Enright, Kubota, Yang, & Tian, 2003; Enright, Sung, Chang, Yang, & Tian, 2005) or cell cycle stage (Campbell, Loi, Otaegui, & Wilmut, 1996; Wells et al., 2003). Another approach is fusing transiently permeabilized cells containing artificially condensed chromatin (Sullivan, Kasinathan, Kasinathan, Robl, & Collas, 2004). Activating with sperm rather than by artificial methods (Schurmann et al., 2006) can have a positive influence on SCNT by providing a better Ca-signaling impulse and contributing some factors (centrosome, mRNAs, microRNAs) to the cytoplasm.

**Genetic and epigenetic properties of clones**

**Genetic aspects**

Cloning by SCNT generates in principle animals genetically identical to the nucleus donor. In practise, the situation is somewhat more complex. Somatic cells are assumed to harbour all the same genome but mutations of some genes which are silent in somatic cells may have occurred during the life of the animals and, strictly speaking, the genomes used for cloning are not completely known.

Difference between clone and the donor animal may also stems from mitochondrial heterogeneity. In sexual reproduction, male mitochondria are eliminated from the oocyte cytoplasm. In SCNT, embryos may contain mitochondrial DNA from the oocyte cytoplasm only or from both the donor cell and the oocyte cytoplasm (mitochondrial heterogeneity). The vast majority of clones analysed so far contained only a small number, if any, of mitochondria from the donor (Hiendleder et al., 2006), although in some exceptional cases a high proportion of donor mitochondria has been detected (Burgstaller, Schinogl, Dinnyes, Muller, & Steinborn, 2007). Yet, it cannot be ruled out that some adult metabolic diseases could result from the presence of donor mitochondria (McConnell, 2006).

Telomeres are short, highly repetitive DNA sequences located at the ends of chromosomes which prevent degradation of chromosomes. An enzyme, telomerase, present in various renewal tissues including germ cells and embryonic cells has the ability to extend, or to hold constant, the length of the telomeres over multiple cell divisions. Telomeres of the first mammalian clone, “Dolly”, were found to be shorter than those of the age-matched, naturally bred counterparts. In sheep similar results have been published showing that clones derived from cultured somatic cells have shortened telomere lengths compared to age-matched controls and donor cell cultures beyond 20 population doublings had significantly (P < 0.05) shortened telomeres (Alexander et al., 2007). The offspring derived from natural mating between clones had normal telomere lengths compared to their age-matched counterparts. However, in later studies, the telomere length in cattle, pig and goat clones were comparable to or even longer than age-matched naturally bred controls, even when senescent donor cells were used for cloning (Betts et al., 2005; Leon et al., 2005; Jiang et al., 2004; Lanza et al., 2000; Schaetzlein & Rudolph, 2005). The telomere length of 30 offspring from the same bull clone was not different from age-matched controls (Ortegon et al., 2004).
Epigenetic aspects

Mammalian genomes contain about 25,000 genes and only 2000 of them are required for the normal activity of a somatic cell. About 1000 genes are expressed in all cell types and are considered as housekeeping genes. The other 1000 genes are responsible for the differentiated state of somatic cells. Gamete formation implies that the 23,000 silent genes of the somatic gonad cells are reactivated as about 10,000 genes are needed for the early embryo development and the other genes for the different cell function in adults. This reprogramming of the genome in somatic cells to obtain gametes occurs during gamete formation but also during the early steps of embryo development.

Genes are active or not according to the presence or the absence of specific inducers. To be active, a gene must be accessible to its inducers. DNA is associated to proteins and particularly histones. According to the chemical status of histones (e.g. more or less acetylated or methylated), chromatin adopts several conformations considered as open or closed, permitting access or not to specific genes of the inducers and the transcription machinery. Moreover, the cytosine base of the CpG structures in DNA may be methylated or not. The DNA methylation state corresponds to inactive genes. Both the histone and DNA biochemical modifications are essential for the regulation of gene expression and known as epigenetic mechanisms. The biochemical modifications of chromatin proteins are a reversible and dynamic process whereas DNA methylation is much more stable, but also reversible. Somatic cell reprogramming consists to a large extent of DNA demethylation followed by specific remethylation of DNA regions which must remain silent in a given cell type. Epigenetic mechanisms affect the expression of some genes and these modifications may be transmitted to daughter cells (Jablonska & Lamb, 2002).

The dedifferentiation of the somatic donor nucleus after SCNT requires changes in DNA and chromatin which are essentially dependent on components found in the cytoplasm of the recipient oocyte. These changes may partially mimic those taking place after fertilization (Jaenisch & Wilmut, 2001). Consequently, the clone embryos often mimic those taking place after fertilization (Jaenisch & Jaenisch, 2006; Kishigami et al., 2006). This suggests that the epigenetic status of embryonic cells forming the inner cell mass is relatively well restored after SCNT at the blastocyst stage. On the contrary, the DNA of trophoderm cells that are the precursors of placenta is excessively methylated (Yang, Smith, et al., 2007). This may explain why about 400 genes out of 10,000 examined showed abnormal expression in the placenta of mouse clones and why this organ is often altered in clones. These changes in DNA methylation patterns have also been observed in in vitro fertilization and embryo culture (without cloning) and in a protocol- and tissue-specific manner, resulting in foetal overgrowth correlated with endocrine changes (Hiendleder et al., 2006).

Not all epigenetic alterations observed in early SCNT embryos result in abnormalities. Hypomethylation of the genes involved in the X-chromosome inactivation process has been observed in various organs of stillborn calves. Although, as no disturbance of sex development has been reported in clones, the implications of the hypomethylation of the X-chromosome observed in dead clones are unclear for healthy clones (Xue et al., 2002). More generally, it must be considered that the two copies of a gene have little chance to be simultaneously epigenetically silenced in a clone. The silencing of specific genes by epigenetic mechanisms or inactivation of a pathway may be compatible with a normal life of the clones.

Although global analysis of the methylated status of clones is lacking in domestic species, a study in swine clones included evaluation of methylation in two different regions of the genome (Archer et al., 2003). Compared to control pigs, clones demonstrated differences in the methylation status in both transcribed and untranscribed regions of the genome, indicating that the cloning process may alter the pattern of DNA methylation. However, because all of the clones in this study were healthy at the time of study (27 weeks of age) and had no apparent developmental defects, the biological relevance of these differences in DNA methylation is unclear.

Study in mouse indicates that, after cloning, epigenetic abnormalities such as those resulting in an obese phenotype are corrected in the germ cells of clones so that the offspring do not exhibit the obese phenotype (Tamashiro, Wakyama, Blanchard, Blanchard, & Yanagimachi, 2000). Many genes with epi-alleles may exist in the genome but their detection requires a visible effect on the phenotype in both the clone and its progeny (Peaston & Whitelaw, 2006). Recent data indicated that 30 offspring generated
by the same bull clone, lost all the abnormalities observed at birth and postnatally in the genitor (Ortegon et al., 2007).

Transgenerational epigenetic inheritance in response to various conditions has been observed in many eukaryotes and may play an important role in mammals (Peaston & Whitelaw, 2006). Environmental influences may induce a number of epigenetic modifications leading to the silencing or activation of specific genes, especially when pregnant females are maintained in conditions resulting in stress in the dam and foetus. The epigenetic modifications observed in the offspring of those pregnancies may then be transmitted to their progeny (Gluckman, Hanson, & Beedle, 2007a, 2007b). Moreover, there is evidence suggesting that RNA can be a determinant of inherited phenotype. In the Agouti phenotype, the white tail tip trait in mouse is not transmitted in a Mendelian fashion but by RNAs packaged in sperm (Rassoulzadegan et al., 2006). No similar studies or outcomes have been identified in the livestock species. The relevance of these observations to clones and their offspring are not entirely clear. One hypothesis is that clones are epigenetically modified animals, with the cloning process itself functioning as the epigenetic inducer. The healthy clones may then be considered as having epigenetic modifications compatible with a normal life. Epigenetic dysregulation is not a phenomenon unique to cloning and has been observed in all other forms of reproduction, but particularly in ART that have a considerable in vitro component. This has been observed in cattle when in vitro fertilized embryos and embryos derived via SCNT were compared to in vivo produced embryos (Camargo, Viana, Sa, Ferreira, & Vale Filho, 2005; Smith et al., 2005). It is not known whether these abnormalities are due to the stress of SCNT per se, or as the result of the in vitro environment that the early embryos are exposed to prior to transfer to the surrogate dam. However, Smith et al. (2005) found that the SCNT bovine embryos’ gene expression profiles were very different from those of their donor cells and very closely resembled those of naturally fertilized AI embryos, more so than IVF embryos, representing the complexity of this issue.

Health and welfare of clones

Animal health is referred to as a state where an animal can sustain its biological function to sustain its own integrity and animal welfare when its physical and behavioural needs are fulfilled which include the absence of pain, distress and suffering. The evidence for poor health and welfare, or improved health and welfare, may change according to the context of the various phases of the animal life. The current available data with reference to clones have been generated mostly by comparing clones with animals that are not clones. It is important, in regard to the risks associated with the cloning technology, to distinguish clearly between the risks directly related to the technology of cloning itself, and those related to the stage of development of the technology and the degree of the control of the processes which are used.

Qualitative and preferably quantitative data are required to assess welfare indicators directly on the animals concerned. Since animal cloning is a relatively recent technology data are still scarce and it is therefore difficult to draw any conclusions on welfare from the limited behavioural studies available (Archer, Friend, Piedrahita, Nevill, & Walker, 2003a, 2003b; Coulon et al., 2007; Savage et al., 2003).

The draft EFSA opinion (EFSA, 2008) considers health aspects in relation to the surrogate dams, to clones and their progeny. For surrogate dams, an increased proportion of pregnancy failure has been observed in cattle and pig and increased frequencies of hydrops and dystocia have been observed especially in cattle (Arnold, Bordignon, Lefebvre, Murphy, & Smith, 2006; Batchelder et al., 2005; Lee et al., 2004). This and the increased size of the offspring (large offspring syndrome; LOS) make Caesarean sections more frequent in cattle carrying a clone than with conventional pregnancies (Constant et al., 2006). These effects have also been observed in surrogate dams carrying pregnancies induced by assisted reproductive technologies (ART) not involving SCNT, albeit at a lower frequency and often with less severity (Farin & Farin, 1995; Walker, Hartwich, & Seamark, 1996). Mortality and morbidity rates in clones are higher than in sexually reproduced animals but most clones that survive the perinatal period in pigs and the juvenile period (up to about 6 months) in cattle are normal and healthy as determined by physiological measurements as well as by behaviour and clinical examinations (Chavatte-Palmer et al., 2004; Heyman et al., 2007; Panarace et al., 2007; Wells, Forsyth, McMillan, & Oback, 2004). There is no evidence indicating adverse outcomes for the sexually reproduced progeny of cattle or pig clones. However, it should be noted that neither clones nor their progeny have yet been studied for their full natural lifespan, and that no studies on the welfare of the progeny of livestock clones have been reported.

The draft EFSA opinion addresses animal welfare and indicates that the cloning procedure itself does in general not affect the welfare of the animals from which the somatic cell nucleus and oocyte are obtained (EFSA, 2008). Reduced welfare of clones is assumed to occur as a consequence of adverse health outcomes. For the surrogate dam carrying calf clone the occurrence of late gestational losses, dystocia and LOS is likely to affect welfare and the frequency of adverse health outcomes is higher in SCNT compared to in vitro or in vivo reproduction. Due to the low efficiency of the cloning process, also a high number of surrogate dams are required to produce a low number of clones which indirectly may affect the welfare of a cohort of surrogate dams. However, the proportion of adversely affected clones could decrease as a result of good animal management and as the technology improves.
In some experiments but not in others, it has been observed that mouse clones have a lifespan shortened by an average of 10% whereas other studies has not seen signs of premature ageing, but that lifespan was shortened due to increased sensitivity to diseases (Wakayama, 2004). It is not known if clones of farm animals have a shorter lifespan as they are still too young, and because farm animals are normally slaughtered long before the end of their natural life.

The European Group on Ethics (EGE) has adopted an Opinion on the request of the President of the European Commission on the ethical aspects of animal cloning for food supply (EGE, 2008). Considering the current level of suffering and health problems of surrogate dams and animal clones, the EGE has doubts as to whether cloning animals for food supply are ethically justified. Whether this applies also to progeny is open to further scientific research. The draft EFSA opinion as well the EGE opinion state that there is a lack of data on the long-term animal welfare and health implications of clones and their offspring, due to the current limited use of the technology. Therefore it is recommended to perform further studies and analyses on long-term animal welfare and health implications for clones and their offspring, as well as more comparative analyses with other assisted and traditional reproductive technologies in animal farming.

Safety of meat and milk from clones and clone progeny

The safety of food from clones and progeny has been examined in several countries in cows and pigs using various criteria.

Composition of meat and milk

Several parameters have been measured in clones and clone offspring in various studies such as gross carcass characteristics, milk volume, water, fat, proteins, carbohydrates, amino acids, fatty acids, vitamins and minerals (Heyman et al., 2007; Norman, Lawlor, Wright, & Powell, 2004; Norman & Walsh, 2004; Shibata et al., 2006; Takahashi & Ito, 2004; Tian et al., 2005; Tome, Dubarry, & Fromentin, 2004; Walker et al., 2007; Walsh, Lucey, Govindasamy-Lucey, Pace, & Bishop, 2003; Yang, Tian, et al., 2007). In one study more than 150 parameters in 37 cow clones from 3 independent groups of clones and 38 control animals were examined over a 3-year period (Heyman et al., 2007). Only a few very slight differences among the 10,000 individual measurements were observed between the clones and their controls, e.g. in fatty acid composition of milk and muscle of clones and a slight increase of stearoyl-CoA desaturase in milk and muscle. These variations were within the normal range of controls and are not expected to have an impact on food safety.

Meat composition data for 5 pig clones and 15 comparators showed no biologically relevant differences in fatty acid, amino acid, cholesterol, mineral and vitamin values. The composition of 242 pig clone offspring, from four boar clones and 162 controls from the same breed were compared (Walker et al., 2007). Among the 58 parameters and the 24,000 individual measurements examined, only 3 individual values of the offspring were different from the normal range of the controls and 2 out of the 3 were within the normal range found in pigs, according to the USDA National Nutrition Database.

Toxicity, allergenicity and genotoxicity of clones

Clones as well as conventional food production animals are unlikely to have genes or biochemical pathways to produce toxins. Toxicity of livestock animals may instead result from indirect effects such as minor changes of protein glycosylation or over accumulation of toxic residues. Evaluating the toxicity of meat or milk is more difficult than for individual compounds as the amount of clone products given to the test animals remains limited by their capacity to eat these products. The evaluation of food products from clones and offspring is therefore based on the concept of substantial equivalence comparing measurements of biochemical parameters and nutritional substances with conventionally reproduced control animals and data found in available databases.

Rats fed for 14 weeks with a diet containing meat and milk derived from embryonic and somatic clones were not affected by the consumption of meat and milk from bovine clones (Yamaguchi, Ito, & Takahashi, 2007). A similar conclusion was drawn with rats fed for 21 days using a diet containing milk and meat of cattle clones (Heyman et al., 2007). Rats fed with milk and meat from cattle clones and controls developed, as expected, a weak immune reaction. The antibodies were in both cases IgG, IgA and IgM but not IgE, indicating that the consumption of the cow products induced a classical immune response but no allergenic effect (Takahashi & Ito, 2004). Intraperitoneal injections of meat extracts and milk into mice were performed and no difference in the allergenic potential was observed between samples from clones and comparator control cattle (Takahashi & Ito, 2004). Similarly, Heyman et al. did not detect differences in the allergenicity of milk and meat obtained from clones or control animals (Heyman et al., 2007). Meat derived from cattle clones did not show any genotoxic potential using the mouse micronucleus assay (Takahashi & Ito, 2004).

In conclusion the available reported studies, and available risk assessments does not indicate that animal clones, their products or offspring from clones would constitute any additional food safety risks compared with their conventional counterparts (EFSA, 2008; FDA, 2008).

Impact on environment and genetic diversity

There is no expectation that cattle and pig clones or their progeny would pose any new or additional environmental risks compared to conventionally bred animals. There is also no information to suggest that such risks may exist.
But it should be noted that there are no studies published on the environmental impact of clones. Cloning does not seem to have a direct effect on genetic diversity but there could be an indirect effect due to overuse of a limited number of breeding animals in breeding programs. An increased homogeneity of a genotype within a population may increase the susceptibility of an animal population to infectious and other risk factors. Cloning offers opportunities to save endangered species or livestock breeds and can be used to restoring populations from infertile or castrated animals (Meirelles et al., 2001; NZRBCS, 2002; Oh et al., 2008). This implies preservation of cells obtained from animals to produce genitors that could be used in subsequent breeding programs to expand endangered populations.

Conclusions

The substantial epigenetic reprogramming taking place of the genetic material of the donor cell consists of several crucial stages. The process at each stage is subject to possible failures that may constitute an epigenetic dysregulation which consequently may lead to failure of the SCNT cloning process. The health of clones can be adversely affected due to the epigenetic dysregulation, and this health effect can also give rise to decreased welfare of animal clones. Epigenetic modifications have also been seen in healthy clones, and are likely to be a normal process in all animals. There have been no health effects seen in sexually reproduced offspring of clones and conventional animals. Meat and food products (such as milk from cattle) from clones and their offspring are within the normal range observed in conventional products, and it is unlikely to be any food safety issues consuming such products.

References


Recent progress in exposure assessment and its interaction with the risk analysis process

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The exposure assessment for chemicals in food is a crucial component of the risk assessment process and is part of the practices of expert committees and panels. Its combination with the toxicological evaluation results in characterisation of the risk for human health.

According to article 22 of Regulation (EC) No. 178/2002, data are needed to permit European Food Safety Authority (EFSA) to carry out its mainstay risk assessment activities. A critical element in the conduct of risk assessments is therefore the exposure assessment which combines data on concentrations of a chemical substance or a biological agent in foods with data on the quantity of those foods consumed across the European Union (EU). The methodology can equally be applied to harmful as well as beneficial substances with regard to food components that are naturally present, food additives, food supplements, contaminants and pesticide residues.

The aim of the present paper is to describe the most recent evolutions and the short-term perspectives for exposure assessment driven by the progress of the risk analysis process itself and to describe the scientific and technical challenges to take into account the exposure assessment as an internal expertise within the EFSA including the creation of databases for food consumption, food composition and food contamination.

Introduction

The risk assessment for chemicals in food exists since the 60s (Truhaut, 1991) but it is only in the middle of the 90s that the estimation of the exposure was included in the practices of expert committees and panels, as a scientific component beside the toxicological evaluation. Since that date, most of the scientific opinions include a risk characterisation based on a comparison between the dietary exposure and the health based guidance values i.e. the acceptable/tolerable daily intake.

The aim of the present paper is to describe the most recent evolutions and the short-term perspectives for exposure assessment driven by the progress of the risk analysis process itself. In addition, in a second part of the article, the organisation of the EFSA to take into account the exposure assessment as an internal expertise including the creation of databases for food consumption, food composition and food contamination will be described.

Initial context for the implementation of exposure assessment

The international assessment of the risk from food became a crucial issue in the middle of the 90s, firstly because of the Agreement on the Application of Sanitary and Phytosanitary Measures (SPS agreement) adopted by the World Trade Organisation (WTO, 1994) and its implementation involving the Codex Alimentarius Commission (CAC) and the related scientific bodies (JECFA, JMPR and latter JEMRA) in the mechanism of regulation of international trade. In brief, the World Trade Organisation encourages Member States to utilise the international standards proposed by the Codex Alimentarius Commission and the CAC develops standards using a risk assessment procedure based on scientific evidences.1

1 “Members shall ensure that their sanitary or phytosanitary measures are based on an assessment, as appropriate to the circumstances, of the risks to human, animal or plant life or health, taking into account risk assessment techniques developed by the relevant international organizations.”
Another important issue which influenced the risk assessment process within the EU was the so-called BSE crisis. One of the follow-up of this sanitary scare was the re-organisation of relationship between risk assessors and risk managers. In particular, all the scientific committees were grouped under the umbrella of the Directorate General “Health and Consumer Protection” (DG SANCO) of the European Commission, facilitating therefore the harmonisation of approaches between the panels dealing with various issues (food additives, contaminants, packaging material ...). In addition, the EU Commission (and later the EFSA) developed and applied a procedure of selection to nominate the members of the scientific committees instead of previous non-homogeneous criteria used by Member States to designate experts. This change in the organisation gave the opportunity for a new generation of scientists to participate in risk assessment activities and for new methods to emerge.

A number of publications exist to describe in detail the exposure assessment process as well as to be used as guidelines for a stepwise approach. The two major references are currently the reports of the EU project FOSIE (Kroes et al.) and the one of the FAO/WHO consultation (WHO, 2005).

New challenges for risk analysis

During the last few years, risk assessors were confronted to a number of new requests to improve the quality and the accuracy of the risk estimates.

Uncertainty analysis

Risk managers are relatively often confronted with scientific opinions concluding on a possible exposure of consumers above the health based guidance values. They therefore need more quantitative estimates in order to know the magnitude and duration of this exposure as well as the size of the over-exposed population. The response of scientists consists in the utilisation of statistical modelling for both hazard characterisation (Benchmark dose approach) and exposure assessment (exposure modelling) in order to characterise the risk as a probability rather than as a possibility. One of the direct consequences of such a stochastic approach is the need to express the uncertainty around the estimations.

The Scientific Committee of the EFSA adopted in 2006 (EFSA, 2006) a very pragmatic and useful opinion on the way to deal with uncertainty in exposure assessment. In this opinion, the Committee proposes a classification of uncertainty: measurement uncertainty, sampling uncertainty, extrapolation uncertainty, model uncertainty, dependencies, imprecise language, disagreement and ignorance. It recommends using a range of uncertainty analysis (qualitative, deterministic or probabilistic) as a function of the type of assessment under consideration.

The uncertainty analysis is certainly a progress for the exposure assessment procedure but also represents an increase of workload for scientific panels. Its systematic implementation in future opinions will depend on the harmonisation of practices between scientific panels and of similar guidelines for uncertainty analysis to be used for the other components of risk assessment. Furthermore a “positive” description of the uncertainty analysis or a mention of the “certainty” of the risk assessment components’ would allow a more balanced communication to risk managers and consumers, raising the fact that current approach is not less robust but certainly more transparent than in the past.

Risk/benefit approach

Historically, the risk assessment applied to food was used to estimate the safety of chemicals intentionally added (Truhaut, 1991). Latter on, the concept of Acceptable Daily Intake was extended to natural and environmental contaminants and named Tolerable Daily Intake. Therefore, because chemical contaminants cannot be totally avoided in food, the assessments requested by risk managers slightly moved from the risk of chemicals to the risk of food itself. One demonstrative example of such a situation was the question of the European Parliament (EP) to the EFSA about the safety of wild and farmed fish (EFSA, 2005a).

It is obvious that considering the current environmental pollution, a regular consumer of high amounts of fish is likely to exceed the Tolerable Intake for some contaminants. Nevertheless, the answer of the EFSA to the EP emphasizes the fact that, from a public health point of view and at the population level, the benefit of eating fish appears to be higher than the potential risk and fish consumption should still be encouraged (EFSA, 2005a). Therefore, it appears that the risk assessment which was usually performed in isolation from nutritional benefits of a considered food category should be replaced by a risk/benefit evaluation.

Considering together xenobiotics and nutrient raised a need for toxicologists and nutritionists to build together a common scale for a balanced judgement. The most sensitive aspects of the risk/benefit analysis are certainly the choice of the dose—response relationship linking the health effect(s) and the intake of food constituents together with a realistic modelling of the actual uptake of these constituents from various food consumption patterns. For an exposure assessment perspective a risk/benefit characterisation also implies to link the databases on food contamination with that on food composition and in a further step with the food consumption data. Up to now almost each Member State of the EU developed databases on food composition (Deharveng, Charrondiere, Slimani, Southgate, & Riboli, 1999; Ireland & Møller, 2006) and/or food consumption (EFCOSUM, 2002). Similarly, many data related to food contamination are collected at national and even regional level. The creation of a European platform collecting, integrating and analysing all these datasets in a unique database is certainly a necessity to ensure that the results of the ongoing work to quantify the relationship between food and health will be implemented in future risk/benefit...
assessments. A detailed description of this process will be
given in the second part of this paper but it is important
to mention that up to now, no international institution had
either the legitimacy to collect national data or the means
to analyse them in a comprehensive and harmonised way.
This is now the case since the EFSA created in 2006 the
DATEX unit in charge of this task for the European Union.

The data collection is not the only need for a risk/benefit
assessment of food consumed and innovative methodolo-
gies should be developed in order to combine the available
data mentioned above. In that respect, several ambitious
European research projects (QALIBRA, BENERIS,
BRAFO ...) are dealing with this question and are explor-
ing different ways like the DALY/QALY approach, the cost/
benefit evaluation or the tools of genomic, proteomic or
metabonomic. We can assume that in the near future, new
competences will be needed in the scientific panels, in par-
ticular bio-statisticians, economists and social-scientists,
both because the methods will become more and more
sophisticated and because the quantity and the quality of
data to be analysed will increase dramatically.

Finally, we have seen that over the last ten years, the
exposure assessment procedure moved from the isolated
chemical to the whole food, from the point estimate to
the probabilistic approach and from the risk assessment to
the risk/benefit evaluation. For the future it will remain nec-
essary to answer another crucial question: is the consump-
tion of a particular food category (e.g. fish or vegetable) the
critical factor from a public health point of view? In that
respect there are epidemiological arguments to consider
that the pattern of consumptions is a more appropriate
parameter to be estimated and confronted with observed
health effects. In particular the regular discrepancy between
case control studies and intervention studies failing to dem-
strate the (negative or positive) effect of a single food
category is quite in favour of a major role for the whole
dietary pattern. In that respect, the study of associations
and substitutions of food and their evolutions based on
available food consumption surveys is certainly of great in-
terest. This will imply, as it is well summarised in the title
of the EFSA Forum, to move from a “safe foods approach”
to “healthy diet criteria”.

Appropriate level of protection (ALOP)

We have already raised the important role of WTO and
the SPS agreement (see supra) in the elaboration of food
standards based on risk analysis process. In addition,
WTO also recommends Member States to determinate an
appropriate level of protection (also called “acceptable
risk”) defined as “The level of protection deemed appro-
piate by the Member establishing a sanitary or phytosanitary
measure to protect human, animal or plant life or health
within its territory.” (WTO, 1994). From a risk assessment
perspective, the ALOP to be used as a threshold in exposure
models could be considered as a food standard established
on toxicological consideration, in opposition with the
ALARA principle which is used actually by risk managers
to set food regulations mainly established on the rejection
of the highest contaminated samples. A regulatory measure
established on the basis of an ALOP would be in line with
the recent trend described above for scientific assessments
aiming to quantify the health impact of food contamination.
This sensible political question is typically an issue for risk
managers. However, in the absence of a clearly defined
acceptable level of risk, the statistical considerations could
be seen as the unique driving force for the exposure thresh-
olds. As an illustration we can recall that the current expo-
sure assessments are usually estimating the probability to
exceed a toxicological (or nutritional) threshold at the
95th percentile of the distribution. In rare cases (like for
pesticide residues), the probabilistic exposure assessment
relies on the 99.9th percentile. These percentiles corre-
spond respectively to probabilities of $10^{-3}$ to $5 \times 10^{-2}$ to
exceed a health based guidance value and to approximately
500,000–25,000,000 EU consumers. These levels are obvi-
ously NOT accepted levels of risk within the EU and are
only driven by the use of a certain type of data, models
and statistics. For many of the chemical contaminants, the
probability for adverse effects in Human is (fortunately)
more likely to be around $10^{-6}$ rather than around $10^{-3}$
based on epidemiological data or dose–response relation-
ship. To estimate such low probability, the naive Monte-
Carlo simulation used currently by default is not the
appropriate tool and other statistical methods should be
developed. It could be noted that other fields of risk assess-
ment (finance, insurance, space technology ...) are using
sophisticated methods like the particle filtering (Cérou,
Del Moral, LeGland, & Lezaud, 2006) to estimate the like-
lihood of very rare events. These methods should be adpat-
ted to food safety issues.

Finally, the EFSA should urge the decision makers to
include an ALOP into the questions submitted to scientific
panels.

Implementation of exposure assessment

within the EFSA

Pre-amble 49 of Regulation (EC) No. 178/2002 of the
European Parliament and of the Council states the follow-
ing: “The lack of an effective system of collection and
analysis at Community level of data on the food supply
chain is recognised as a major shortcoming issue”. The
pre-amble continues to say that a system for the collection
and analysis of relevant data in the fields covered by the
European Food Safety Authority (EFSA) should be set up
in collaboration with EU Member States.

According to article 22 of Regulation (EC) No. 178/
2002, data are needed to permit EFSA to carry out its main-
stay risk assessment activities. Once the hazard has been
identified and described, public exposure to the hazard is
assessed and contrasted against a toxicological characterisa-
tion of the risks posed by ingesting the hazard. A critical
element in the conduct of risk assessments is therefore the
exposure assessment i.e. the estimation of the likelihood of the consumer to be exposed to a hazardous substance and to quantify exposure. Typically, exposure assessments combine data on concentrations of the exposure to a chemical substance or a biological agent in foods with data on the quantity of those foods consumed across the European Union (EU). The methodology can equally be applied to harmful as well as beneficial substances with regard to food components that are naturally present, food additives, food supplements, contaminants and pesticide residues.

No exposure assessment is more accurate than the precision of the individual constituents on which it is based and the compatibility of the origin of the information. To calculate exposure by combining contaminant data from salmon caught in a pristine river stream in the north of Sweden with amounts of fish consumed in Spain is going to provide misleading results. Equally, combining occurrence of polycyclic aromatic hydrocarbons in dried sultanas from Greece with overall consumption of fruit in Poland is not going to be helpful. Yet, this is the challenge faced by EFSA in calculating Europe-wide exposure scenarios. This challenge can be addressed in two ways. On one hand a worst-case scenario can be calculated and if, when compared with Tolerable Daily Intakes or a Benchmark Dose lower limit at a Benchmark Response of 10% (BMDL10 see EFSA, 2005b for explanation), there is a sufficient safety margin, no further elaboration is required. On the other hand should there be potentially at risk populations more precise calculations will be necessary.

To respond to the challenge, EFSA in 2006 set up the Data Collection and Exposure Unit, DATEX for short. The DATEX Unit, in a collaborative effort with all the EU Member States, is charged with collecting detailed information on food contaminants, food consumption and food composition in support of the food safety risk assessment activities of several of the EFSA Panels. Food consumption information should in the future also be able to be used for pesticide risk assessments and food additive intake, while food compositional information can be used in assessing nutrient profiles of food to determine eligibility for nutrient or health claims and for nutrient intake assessments.

Several steps are necessary to improve the usefulness and applicability of the information collected. Sample selection must be standardised and coverage of geographical regions extended. Laboratory analytical methods must be fit for purpose with adequate sensitivity. Reporting of results should be harmonised and a standardised food description system should be used. Common data interchange formats should be developed to facilitate communication. Methods for capturing food consumption information should be equivalent so that exposure assessment results can be compared across country borders.

Sample selection and geographical coverage

The European Commission has already gone a long way to address sampling by introducing legislation for contaminant testing. However, data for compliance checking are very different from data required for an exposure assessment. Sampling for checking of legislative compliance is often targeted to expected problem areas, while ideally sampling for exposure assessments should be random. There is frequently a focus on products known to be carriers of specific contaminants and results will thus be biased towards the higher end. Not all Member States have the resources or inclination to prioritise testing of all relevant contaminants; so geographical coverage will be imperfect.

A coordinated effort is needed to better standardise and describe sampling. It is possible to also use results from targeted sampling as long as the reason for sampling is clearly stated. Models can be developed to correct for sampling bias as long as a representative sample set is also available. Ad hoc data requests will often result in submission of data acquired for other purposes. To address such sample bias EFSA can issue prospective sample requests. Two such recent examples cover data collections for furans and for acrylamide. Member States have been asked to allocate resources to test for furans and acrylamide in prepared foods after heating. EFSA could also better identify data gaps and encourage testing in a wider geographical region. With the free flow of food products across Member State borders there is already a possibility that, through improvements to labelling of the place of origin for trace back, coverage could be improved.

Analytical methods for contaminants

Regulation No. 882/2004 on official controls performed to ensure the verification of compliance with feed and food law, establishes the need for:

- community Reference Laboratories (CRLs) responsible for providing the National Reference Laboratories (NRLs) with details of analytical methods for the analysis of food and feed, including reference methods;
- multi-annual national control plans that cover food and feed law; and
- Member States to present an annual report to the Commission with information on the implementation of multi-annual national control plans; and an annual Commission report which is to be submitted to the European Parliament and to the Council and made available to the public.

The objective of the CRLs is to improve the quality, accuracy and comparability of results derived by official control laboratories involved in monitoring and control activities. In order to achieve this goal the CRLs are — in close collaboration with the national reference laboratories (NRLs) — working on the development of analytical control guidelines to harmonise the requirements concerning method validation and quality assurance systems. The CRLs are also organising laboratory proficiency tests to ensure high quality in analytical results.
The sensitivity of analytical methods is often modified to fit the purpose of the testing. Thus for compliance testing it is sufficient that the test method has a limit of detection that can identify violations of legislative maximum limits with sufficient accuracy. The limit of detection for exposure assessment purposes must be much lower and close to best practice for samples below the limit of detection to not unduly influence exposure calculations. This will be considered in discussions with Member States to see if the dual purpose of compliance and exposure testing can be accommodated.

Methods for capturing food consumption

To permit the conduct of risk assessments for chemical and biological agents potentially present in food, consumption levels of various food commodities in the EU are required at both aggregated and individual food level. This concerns food consumption by the general population as well as by critical groups that are vulnerable or are expected to have significantly different exposures, e.g. infants, pregnant women, immuno-compromised individuals or the aged. It is further important to be able to estimate low and high percentile consumption levels from information stored in the databases to identify vulnerabilities due to eating habits. Low consumption levels are important for essential nutrient intakes while high consumption levels are important for hazardous agents.

The designs used to collect food consumption data vary significantly depending on, e.g. what is measured, what the level of aggregation is, the observation period, and how the data are obtained. Furthermore, food consumption data obtained at national level can often also not be compared directly because of varying survey methodologies, varying clustering of age groups and differing food categorisation systems used.

The most important data are the quantities of various consumed foods. Food frequency surveys are used to give insight into the frequency with which the listed food items are consumed during a specific time period (e.g. daily, weekly ...) but usually they do not give the number of eating occasions. A 24 h recall or a one or more day dietary record are needed to provide more detailed information such as source of food, time of day and place that foods are consumed. Dietary information can be collected at three different levels of aggregation: national food supply data, data at the household level and data at the individual level. Food supply data or food balance sheets provide gross estimates of the type and amount of food available for human consumption within a country. Such data can be obtained through the World Health Organisation Global Environment Monitoring System — Food Contamination Monitoring and Assessment Programme, commonly known as GEMS/Food or from Eurostat. Household budget surveys also assess food availability, rather than food eaten, as they do not consider food wasted. In addition, they do not consider food consumed outside the household but can supply information on food patterns in subgroups of households. The Data Food Networking (DAFNE) has developed a databank on food availability at the household level. It includes data from 75 surveys in 16 European countries (15 EU Member States and Norway) (Trichopoulou & Lagiou, 1997). Food consumption data collected at individual level facilitate the assessment of exposure since they provide information on food intake and their distribution in well-defined groups of individuals. These data are also understood to more closely reflect actual consumption (Kroes et al., 2002). The most accurate reflection of the actual amounts consumed is the duplicate diet method where a duplicate portion is prepared and weighed. However, the intervention is quite severe and cumbersome and is not commonly used for general consumption surveys.

Methods to collect dietary intake data at the individual level can be subdivided depending on the length of the observation period into short-term and long-term studies. Short-term dietary assessment methods collect dietary information on current intake. They range from recalling the intake from the previous day (24 h recall) to keeping a record of the intake of food and drinks over one or more days (dietary record). Long-term dietary assessment methods collect information on usual food intake over the previous months or years (dietary history or food frequency questionnaire). Each method has its own strengths and weaknesses, and there is no single ideal method.

They can all give slightly different results and when attempting to combine data from different sources standardisation of methodology is very important. EFSA is currently working with Member States to identify the methodology to be used in a future pan-European food consumption survey to provide for the necessary harmonisation and linking to existing initiatives like the completed European Food Consumption Survey Method (EFCOSUM) project (Brussaard et al., 2002) and its follow on project — the European Food Consumption Validation (EFCOVAL) project, which started in 2007.

Food description

Calculation of exposure based on contaminant and consumption information requires precise identification of foods to be able to match the two data sources. Even data of good quality can be a source of error if they are derived from foods that are not clearly defined. It is difficult to understand, compare and use data from a multitude of sources without a coherent description of the food products. Names used for a food by the local population are often inadequate or even misleading to those who are not closely acquainted with the local language and culture. A common name may be used for different foods in different regions (Ireland & Møller, 2006).

The problem of food terminology is not the difficulty of finding the best terms or the best ways of classifying foods, but the fact that differing, inconsistent, and incompatible terminologies are used to satisfy the immediate
requirements of the scientific task. Because of the different
descriptions, it is difficult to exchange data between coun-
tries, between organisations within the same country, or
even between workers in the same institution. Even in
EFSA there are several different description systems in
use because of different legal requirements. The pesticide
legislation specifies one system different from the addi-
tives legislation. Foods in the concise food consumption
database have been classified according to the Eurocode
2 food terminology useful to dietary studies (Unwin,
2000).

Harmonisation of food classification, or at least develop-
ment of a translation layer between the different description
systems, is a priority task for EFSA. To harmonise food
recognition a uniform food classification system should
be adopted to group or aggregate foods with similar charac-
teristics into universal food groups. However, since oppos-
ing systems already exist to fulfill requirements set by
different regulatory/legal bodies, it will be necessary to
also develop or adopt a food description system at local
level to identify the food as precisely as possible, without
the need for aggregation. The food description layer will
serve as a translation tool between opposing classification
system and will facilitate the food grouping process. One
such system is LanguaL (Møller & Ireland, 2007). EFSA
is currently supporting a project funded through the Article
36 mechanism of the European Parliament and Council
Regulation (EC) No. 178/2002 to examine the feasibility
of modifying and adopting LanguaL for data transfers
between EFSA and Member States.

Common data interchange formats

In undertaking its work EFSA is dependent on submis-
sions of chemical contaminant data from Member States
and industry to cover as much as possible of the food con-
tamination in the European Union. Several calls for infor-
mation are issued each year for data on specific
substances. Many Member States have data repositories
for storing chemical contaminant information, but the informa-
tion is stored in the local language and the associated in-
formation collected to describe the product and the testing
will vary widely between countries. In merging the informa-
tion from several different sources it is crucial for
EFSA to be provided with sufficient descriptive informa-
tion about the analytical methods used, the food products
tested, the circumstances of sampling, and the precision
and expression of the numerical results, all in a common
language that can be easily combined into one central
repository.

The central repository can be fed manually, relying on
the goodwill of organisations in the Member States to tran-
scribe the information stored locally into a suitable trans-
port format, currently often Microsoft Excel spreadsheets
in an attempt to make data collection and submissions as
easy as possible. However, data precision details and meta-
data describing the survey information are often missing or
inaccurate and much detail is often provided in the local
language. Data modification, quality control and validation
crucial for the incorporation of the data into the overall
material are thus time consuming tasks for EFSA staff.

As an alternative, EFSA is currently pursuing a data col-
lection framework project to streamline data transfer
between local information storage and a generic central
repository. The streamlined system could rely on a mixed
manual intervention model with some automated checking,
but EFSA would also like in the future to explore a model
where data is automatically accessed from decentralised
Member State repositories using a data warehousing archi-
tecture. Particularly for the latter model to work, adoption
of a common food classification and sample description
system across EU Member States will be essential. Such
a system would also greatly improve the efficiency of the
manual intervention model.

Standards should be defined for describing analytical
methodology including limits of detection and quantifica-
tion, and any other barriers in clear results transfer includ-
ing the numerical expressions used.

Concluding remarks

The present paper was prepared in the context of the
fifth anniversary of the European Food Safety Authority
and gave the opportunity to make a point about the progress
of exposure assessment as a crucial and relatively new com-
ponent of the risk assessment process.

It is clear that the creation of the EFSA gave a signifi-
cant impulsion to the harmonisation of data collection
across the EU Member States even if scientific and tech-
nical difficulties remain. During the last years, the Euro-
pean Union implemented, with the scientific support
from EFSA, highly protective criteria for the safety of
food. The challenge for the scientists involved in the
EFSA activities in the future will be to assess and to
quantify the impact of the whole diet(s) on public health
and to provide guidance to risk managers for possible pre-
vention of illnesses. In this context the surveys of food
consumption together with the analysis of food composi-
tion and food contamination will represent essential tools
either to assess the risk or to evaluate the impact of regu-
larly measures.

Appendix. List of abbreviations

JECFA Joint FAO/WHO Expert Committee on Food
Additives and Contaminants
JMPR Joint FAO/WHO meeting on pesticide residues
JEMRA Joint FAO/WHO on Microbial Risk Assessment
BSE bovine spongiform encephalopathy
FOSIE FOod Safety In Europe
FAO Food and Agriculture Organisation
WHO World Health Organisation
QALIBRA http://www.qalibra.eu/overview/

BRAFO http://europe.ils.org/activities/ecprojects/BRAFO/

DALY disability adjusted life years; QALY quality adjusted life years

References


Active and intelligent food packaging: legal aspects and safety concerns

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Active and intelligent (A&I) food packaging based on deliberate interaction of the packaging with the food and/or its direct environment. This article presents: (i) the main types of materials developed for food contact; (ii) the global market and the future trends of active and intelligent packaging with a special emphasis on safety concerns and assessment; and (iii) the EU Legislation and compliance testing of these novel food packaging technologies.

Introduction

New food packaging technologies are developing as a response to consumer demands or industrial production trends towards mildly preserved, fresh, tasty and convenient food products with prolonged shelf-life and controlled quality. In addition, changes in retailing practices (such as market globalisation resulting in longer distribution of food), or consumers way of life (resulting in less time spent shopping fresh food at the market and cooking), present major challenges to the food packaging industry and act as driving forces for the development of new and improved packaging concepts that extend shelf-life while maintaining and monitoring food safety and quality.

Traditional food packaging is meant for mechanical supporting of otherwise non-solid food, and protecting food from external influences, like microorganisms, oxygen, off-odours, light etc. and, by doing so, guaranteeing convenience in food handling and preserving the food quality for an extended time period. The key safety objective for these traditional materials in contact with foods is to be as inert as possible, i.e., there should be a minimum of interaction between food and packaging.

In the last decades, however, one of the most innovative developments in the area of food packaging is the ‘active and intelligent’ (A&I) packaging, based on deliberate interactions with the food or the food environment.

The purpose of the ‘active packaging’ is the extension of the shelf-life of the food and the maintenance or even improvement of its quality. While the purpose of ‘intelligent packaging’ is to give indication on, and to monitor, the freshness of the food.

Due to its deliberate interaction with the food and/or its environment A&I packaging poses new challenges to the evaluation of its safety as compared to the traditional packaging. However, also in this case the migration of substances from the packaging to food is the main risk. Other risks could occur from the incorrect use of the packaging due to the insufficient labelling or the non-efficacious operation of the A&I packaging. The Regulation 1935/2004 on materials and articles intended to come into contact with food already contains general provisions on the safety of active and intelligent packaging and sets the framework for the EFSA’s safety evaluation process.

A new Regulation specific to (A&I) packaging is expected to be published in 2009 with provisions dedicated to this type of packaging. The EFSA’s guidelines on the
data to be provided for safety evaluation are currently under preparation to help risk managers define lists of authorised substances.

Many scientific publications and reviews have been recently devoted to research on active and intelligent food packaging technologies (e.g. Alvenainen, 2003; Brody, Strupinsky, & Kline, 2001; Chielinii, 2008; Han, 2005; Ozdemir & Floros, 2004; Wilson, 2007). The objective of the present article is to give an overview of history, main types and principles, global market and future trends, with a special emphasis on safety concerns, legal aspects and compliance testing of these novel food packaging technologies.

Technological evolutions and safety evaluation of active and intelligent food packaging
Definitions and brief history

Innovations in packaging were up to now limited mainly to a small number of commodity materials such as barrier materials (new polymers, complex and multilayer materials) with new designs, for marketing purposes. However, food packaging has no longer just a passive role in protecting and marketing a food product. New concepts of active and intelligent packaging are due to play an increasingly important role by offering numerous and innovative solutions for extending the shelf-life or maintain, improve or monitor food quality and safety (Gontard, 2006). Various terms are used for describing new packaging technologies, e.g. ‘active’, ‘interactive’, ‘smart’, ‘clever’, ‘indicators’ etc (Fabech et al., 2000; Gontard, 2000).

Two definitions are now included in the new Framework Regulation on Food Contact Materials (1935/2004).

(i) ‘Active food contact materials and articles’ are defined as materials and articles that are intended to extend the shelf-life or to maintain or improve the condition of packaged foods. They contain deliberately incorporated components intended to release or absorb substances into or from the packaged food or from the environment surrounding the food. Active materials and articles are allowed to bring about changes in the composition or the organoleptic characteristics of the food on condition that the changes comply with the provisions foreseen in the Community or national food legislations.

(ii) ‘Intelligent food contact materials and articles’ are defined as materials and articles that monitor the condition of packaged food or the environment surrounding the food.

Paradoxically, whereas the concept of active and intelligent packaging is now considered as ‘modern’, the concept belongs to ancestral traditions in all tropical areas of the world.

In these regions of Africa, Asia and South America, vegetal leaves were, and still are, profusely and traditionally used for food packaging with important markets dedicated to leaves commercialization. Beyond their use as a simple ‘barrier’, numerous varied vegetal leaves are used for their ability to transfer to foods, aromatic, coloring, enzyme (e.g. papain) or antimicrobial substances (e.g. essential oils). Vegetal leave packaging interacts with foods for modifying their texture, organoleptic properties or slowing down microbial spoilage. They are also used for their ability to change colour with temperature and/or time thus playing the role of cooking or freshness indicators. Up to four different types of leaves are used as successive layers, each of them having a very specific function. Leaves have also been used for ages in Mediterranean regions of Europe, e.g., to wrap traditional cheeses for allowing good maturation process.

In developed countries, moisture and oxygen absorbers were among the first series of active and intelligent packaging to be developed and successfully applied for improving food quality and shelf-life extension (e.g. for delicatessen, cooked meats etc.) Next to these, numerous others concepts such as ethanol emitters (e.g. for bakery products), ethylene absorbers (e.g. for climacteric fruits), carbon dioxide emitters/absorbers, time/temperature and oxygen indicators etc. have been developed (Fig. 1). In a general way, the field has been extended largely as a series of niche markets owing to the current approach of packaging industries looking at it in terms of new market opportunities (Rooney, 2005). The rationale in considering a range of packaging options (both passive and active) as a whole and as a part of the food technological itinerary has not yet been commonly adopted (Gontard, 2006).

If compared to Japan, USA or Australia, the penetration of active and intelligent packaging in the European market is limited thus far. This time lag compared to Japan, USA and Australia was mainly attributed to an inadequate and not enough flexible European regulation that could not keep up with technological innovations in the food packaging sector (Fig. 1). European legislation has been changed in 2004 with the new FCM Framework Regulation (1935/2004) and this change will be soon achieved with a specific regulation entirely devoted to active and intelligent food contact materials. But in addition, it should be mentioned that the issues of acceptance by user industries as well as the more conservative behaviour of European consumers regarding innovations in food, are key points that still need to be addressed in this field. Moreover, in some cases, efficacy, safety and benefits need to be better demonstrated and weighted to increase the number of products launched onto the European market.

Active packaging

The principles behind active packaging are based either on the intrinsic properties of the polymer used as packaging material itself or on the introduction (inclusion, entrapment etc.) of specific substances inside the polymer (Gontard, 2000).

The intrinsic properties of the polymer can give rise to the active function next to, e.g., the intentional grafting of an active group or through the introduction of an active
monomer inside the polymer chain. An active agent can be incorporated inside the packaging material or onto its surface, in multilayer structures or in particular elements associated with the packaging such as sachets, labels or bottle caps.

The nature of the active agents that can be added is very diverse (organic acids, enzymes, bacteriocins, fungicides, natural extracts, ions, ethanol etc.) as well as the nature of the materials into which they are included such as papers, plastics, metals or combinations of these materials.

The active systems can be placed outside the primary packaging, in between different parts of the primary packaging or inside the primary packaging. In this last case, the systems can be in contact only with the atmosphere surrounding the food, in contact with the food surface or placed inside the food itself (for liquid foods). This diversity accounts for the innovation potential in this field but it also represents a real challenge for the safety assessment.

Active packaging can be classified into two main types: non-migratory active packaging acting without intentional migration, and active releasing packaging allowing a controlled migration of non-volatile agents or an emission of volatile compounds in the atmosphere surrounding the food (Fig. 2).

Non-migratory active packaging is a packaging which elicits a desirable response from food systems without the active component migrating from the packaging into the food. The most well known examples of non-migratory active packaging are moisture absorbers, mostly based on the adsorption of water by a zeolite, cellulose and their derivatives etc. The tendency in the market of moisture absorbers systems is to introduce the absorbing substances inside the packaging material in order to make the active system invisible for the consumer such as, e.g., absorbing trays for fresh meat or fish.

Other well known active packaging systems are oxygen absorbers, mostly based on iron oxidation but they can also be based on ascorbic acid or catechol oxidation, on enzymatic catalysis as well as on many other reactions. The development of oxygen scavenging systems was first based on self-adhesive labels, others adhesive devices or loose sachets to be included in the packaging with the food. A second concept, developed later, was based on the design of active substances for being included in the packaging material itself, using monolayer or multilayer materials or reactive closures liners for bottles and jars (Rooney, 2005). Ethylene scavengers, based on various re-actions, are extensively used to slow down the maturation rate of climacteric fruits, a crucial point for import-export of fresh fruits and vegetables. Another example of non-migratory packaging is the antimicrobial packaging based on the entrapment in a silicate network of silver ions, a widely marketed antimicrobial agent. Nevertheless, even food contact antimicrobial systems with an assumed non-intended
migration (e.g. silver or silver based systems, other immobilised or grafted biocides), are known to exhibit some degree of migration. Thus, there is a strong need to better understand the active agent principles and mechanisms as well as to optimise their use in order to design active packaging elements that (i) are sufficiently effective and reduce detrimental side effects and (ii) permit an accurate, knowledge-based, assessment of potential risks. It is clear from the existing literature in the challenging field of antimicrobial packaging that research results on antimicrobial systems are often not reproducible or even contradictory, i.e. the case of chitosan or chitosan-based systems of which systems are often not reproducible or even contradictory, i.e. the case of chitosan or chitosan-based systems of which biocide phenomenology and the optimum efficiency have just recently been understood on the basis of the positive biocide phenomenology and the optimum efficiency have i.e. the case of chitosan or chitosan-based systems of which.

Research in the area of antimicrobial food packaging materials has significantly increased during recent few years as an alternative method for controlling microbial contamination of foods by the incorporation of antimicrobial substances in or coated onto the packaging materials. Much research is devoted to the design of antimicrobial packaging containing natural volatile antimicrobial agents for specific or broad microbial inhibition depending on the nature of the agents used or on their concentration. Different types of antimicrobial delivery systems and packaging material—food combinations are developed to maximize the efficacy of the system.

In the field of active releasing packaging, one of the best-known products is an ethanol releaser which is able to slow down mould growth and thus increasing the shelf-life of bakery products. Water-vapour releasing sachets are a key element of successful innovative packaging for ready-to-cook fresh vegetables. Combined with a valve placed onto the plastic pouch, they permit to cook fresh vegetables in a microwave oven and to obtain, in only a few minutes, a freshly cooked meal with improved organoleptic and nutritional quality.

Another example of active releasing materials is a plastic film commercially available in Japan, containing allyl isothiocyanate (AITC), a strong antimicrobial substance extracted from mustard or wasabi. AITC is entrapped in cyclodextrins for protecting the volatile active agent from being thermally degraded during extrusion. When exposed to high moisture conditions after the packaging of the food product, cyclodextrins have the ability to change in structure and to release the antimicrobial agent in the atmosphere surrounding the food.

In active packaging, nanotechnology has a great interesting potential because nanostructures display a high surface-to-volume ratio and specific surface properties. Nanotechnologies such as nano(bio)composites and electro spin nano-fibre based structures are able to enhance desired properties or to introduce new additional effective functionalities with small amount of nanofilbers. Recent studies also show that nano-particles can be tailored for both controlled release and/or specificity in the action of the active agent having moisture or temperature as triggering mechanisms (second generation of nano-structured materials).

As for any other new technology that is coming into the stage of application, a sound evaluation and a risk-benefit analysis needs to be carried out. In this context, nanostructures are generated by one- or two dimensional nano-additives being the other dimension more in the conventional micro size, thus resulting in a reduced potential mobility.

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**Fig. 2.** Scheme of the two different types of active food contact materials (FCM) classified as a function of intentional or unintentional migrations.
Therefore, these nano-carriers can be considered of less safety concern than other nano-particles. On the other hand, the high surface developed by nano-particles can potentially bind unwanted substances that could be otherwise released in contact with foods. The full understanding of the effect on food safety of the introduction of nanostructures has still to be ascertained.

Intelligent packaging

Intelligent packaging systems attached as labels, incorporated into, or printed onto a food packaging material offer enhanced possibilities to monitor product quality, trace the critical points, and give more detailed information throughout the supply chain (Han, Ho, & Rodrigues, 2005). Intelligent tags such as electronic labelling, designed with ink technology in a printed circuit and built-in battery radio-frequency identity tags, all placed outside the primary packaging, are being developed in order to increase the efficiency of the flow of information and to offer innovative communicative functions. Diagnostic indicators were first designed to provide information on the food storage conditions, such as temperature, time, oxygen or carbon dioxide content, and thus, indirectly, information on food quality, as an interesting complement to end-use dates for example (Fig. 3).

Based on polymerisation rate, diffusion, chemical or enzymatic reactions, the most commonly used of these visual indicators are critical temperature indicators, time/temperature indicators and leak indicators. This first generation of indicators can be considered as “indirect indicators” of food freshness. The trend in this field is to develop direct indicators of food quality because of their ability to provide more precise and targeted information on quality attributes (Gontard, 2004). Targeted quality markers can be volatile compounds (e.g. volatiles of microbial origin) such as carbon dioxide, nitrogen compounds etc. or biogenic amines, toxins as well as pathogenic bacteria themselves. Commercially available direct indicators are, e.g., a pear maturation indicator based on the detection of a volatile aroma compound or a fish freshness indicator based on the detection of volatile amines. More sophisticated systems are based on depositing, on the bar code, a plastic layer loaded with specific antibodies of pathogenic microorganisms such as salmonella or listeria whose presence can be detected when the bar code is read.

Despite active research in the area, the application of intelligent systems in Europe has mainly been limited to the use of visual temperature or time—temperature indicators. Reasons for this may have been the too high cost of an indicator label, legislative restrictions and even acceptance of retailers and brand owners. The fear could be that indicators would reveal possible irregularities occurring, e.g., in the management or control of the cold chain. On the other hand, a few shortcomings of these indicators also exist, e.g. their response to oscillating temperature patterns (Malcata, 1990). Safety issues come mainly from direct indicators that are required to be placed inside the primary packaging for direct contact with the atmosphere surrounding the food or with the food itself, because of potential undesirable migration of chemical components.

Future trends and safety issues

Research and development in the field of active and intelligent packaging materials is very dynamic and develops in relation with the search for environment friendly packaging solutions. In this context, the design of tailor-made packaging is a real challenge and will imply the use of reverse engineering approach based on food requirements and not anymore only on the availability of packaging materials. Nanotechnologies are expected to play a major role here, taking into account all additional safety considerations and filling the presently existing gap in knowledge.

![Fig. 3](https://example.com/f3.png) Food quality indicators: main indirect indicators and trend towards direct indicators of microbial growth and spoilage and others food qualities attribute.
They will be involved in the development of triggered/controlled release of active agents and for targeted indicators. New non-migratory materials for innovative functions such as in-package food processing are also a promising field of development.

In a general way, the answer to safety issues in relation with active and intelligent packaging should be based on three main pillars:

1. Labelling, with the aim to prevent misuse and misunderstanding by the downstream users or consumers, e.g. to avoid sachets from being ingested;
2. Migration of active and intelligent substances should be carefully considered as well as all breakdown products, as function of their toxicity. The compliance of releasing active packaging with food legislation shall be closely related to migration phenomena. Assessing migration implies to develop dedicated migration tests as well as mass transfer modeling tools because those existing, or recommended for conventional plastics, are not adapted to active and intelligent systems;
3. Efficacy of the packaging: finally, in some very specific cases, the ability of the packaging to perform the claimed function can raise safety issues as for any food preservation technology, e.g. delivering a preservative or absorbing oxygen in a suitable way for preventing microbial growth without inducing antimicrobial resistance or pathogen over growth, or giving reliable information on pathogenic bacteria presence for direct indicators.

A flexible legislative framework and appropriate testing methods are required for supporting such a highly innovative field, i.e. to keep up not only with the market as it is, but also as it will be.

**Evaluation of the global market of active and intelligent packaging**

Active and intelligent packaging materials and articles were firstly introduced in the market of Japan in the mid 70s, but only in the mid 90s they raised the attention of the industry in Europe and in the USA. In this period, there was a proliferation of patents and market tests that created great expectations of commercial growth.

In 2002, the global market accounted for about 1.4 M US$, more than 80% of which was represented by two specific active concepts, namely oxygen scavengers and moisture absorbers (40% each), the main application being in areas like optical devices, electronic tools, medical and pharmaceutical preparations and others. Packaging, in particular food packaging, represented a very small fraction of the market in these years and it was almost totally concentrated in Japan. However, the food packaging sector was identified as the most fast growing market; that belief was caused by strong expectations of growth of centrally prepared perishable foods in Modified Atmosphere Packaging (MAP), considered as an ideal field for oxygen absorbing packaging applications.

The 1.4 M US$ value of the market in 2002 was foreseen to attain a value between 2.6 and 3.7 M US$ in 2007 thanks to the development of the bottled bier and fruit juices market, as well as the development of MAP for fresh meat, ready meals, fresh pasta etc. Oxygen scavengers were then believed to reach sales as high as 5.7 billion units in Europe in 2007 (versus 0.3 billion in 2001) and 4.5 billion units in the USA in the same year (versus 0.5 billion in 2001). Although price was already considered one of the main limiting factors, in 2002 almost 80% of the industry was convinced that the technical progress would have decreased it to an affordable level within 2005.

In fact, these optimistic forecasts have only partially been achieved: both Europe and the USA did not fully match the expected growth, while Japan showed a steady market increase in line with the previous years. Although not boosted, the market of centrally food packed in MAP did show an increase. But, in reality, food industries have been somewhat reluctant to invest in active packaging concepts.

There are two main reasons that would explain such behaviour. The first is related to cost: being highly specialised and containing often sophisticated technologies, active and intelligent materials still account for 50–100% of the whole packaging cost. This is simply not affordable by most of the food industries, where packaging cost can hardly exceed 10% of the total cost of items placed on the market, and where special packaging performances may be desired provided that they are supplied at commodity price (this may be less critical for other markets like medical packaging where the packaging cost is proportionally less important). The second reason is acceptance: often consumers do not perceive active and intelligent materials as a strong benefit. Consumers’ attitude is often driven by the idea that food with shorter shelf-life is fresher and a concept like oxygen scavengers that would extend the shelf-life may not be seen attractive, although convenient in certain situations but at the detriment of freshness. Also, time/temperature indicators that provide quite accurate information about the real freshness of the food, or whether the food underwent stressing temperature conditions may not be seen by retailers as a valid sales incentive as they would likely induce consumers to choose only newly displayed items and would increase the amount of unsold foodstuffs.

In a study carried out by PIRA International, both brand owners and packaging converters identified as main resistance to the introduction of these materials in the market the fact that the existing materials were considered already adequate to the market needs (Table 1) (Anonymous, 2006).

As already outlined, price was also seen as a strong limiting factor and overall the lack of perceived consumers’ demand did result into a negative cost/benefit analysis. On the other hand, the perspective of the companies providing the technology leading to these materials was rather
different. They identified as main limiting factor the oftenquestionable efficiency of active and intelligent materials. In fact, in most of the numerous patents filed during the course of the years active and intelligent concepts were demonstrated to perform efficiently almost always in in-vitro essays and under laboratory tests conditions. However, when used in real food packaging applications, they either showed a more limited activity or no activity at all. The rationale of such behaviour lies in the often very different conditions between model tests and real food, e.g. different quantity of foodstuffs packed, ratio and distribution of fat and non-fat parts, fluctuation and variability of physical and chemical parameters such as water activity, pH etc. Another concern, which is perceived as a threat by technology providers, is the lack of a clear regulatory picture, leading to a certain degree of reluctance from food packers to adopt concepts that are not fully covered by the food contact materials legislation. A partial answer to such a concern has been given by the European Commission through the Regulation 1935/2004/EC, which is the Framework Regulation (EC) 1935/2004 for all food contact materials. Under this regulation, there are some specific Directives, e.g. the plastic Directive 2002/72/EC. All food contact materials made of plastic are subjected to this Directive. However, many active and intelligent packaging systems are composed of different materials.

Legislation and compliance testing of active and intelligent food packaging

Legislation

Framework Regulation

To be on the European market, each system should comply with the legislation. As regards the non-active part, e.g. the packaging of the active constituents, it should comply with the applicable food contact legislation (Fig. 1). This is the Framework Regulation (EC) 1935/2004 for all food contact materials. Under this regulation, there are some specific Directives, e.g. the plastic Directive 2002/72/EC. All food contact materials made of plastic are subjected to this Directive. However, many active and intelligent packaging systems are composed of different materials.

Table 2 shows the market value of the main active packaging concepts in 2001 and 2005, together with the forecast for 2010 (Anonymous, 2007).

Besides the interesting result of susceptors and self-venting (450 M US$ in 2005), the numbers of antibacterial films are worth noting. Antibacterial films may find application in the food area, but their market remains almost totally confined in Japan. They represent an example of the heavy influence that regulatory issues may exert over the market of these materials: use of antibacterial films in food packaging has been always put in relation with relaxed hygiene measures, which may be then compensated by the released antibacterial, therefore, perceived as a tool hindering adoption of HACCP by food industries. As a result, no market exists for antimicrobial films in food packaging.

However, not all active and intelligent packaging concepts show just steady market behaviour: there is at least one system the trend of which boosted in the last five years, and that is Radio-Frequency Indicators (RFID). However, it is questionable whether RFID can be classified as active or intelligent food packaging materials, as they do not precisely match the definition of “interacting with food or with the atmosphere to which food is exposed, or giving information on the conditions to which food is or has been exposed”.

Legislation and compliance testing of active and intelligent food packaging

<table>
<thead>
<tr>
<th>Type of A&amp;I system</th>
<th>2001</th>
<th>2005</th>
<th>2010 (Forecast)</th>
<th>Market share (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen scavengers</td>
<td>371</td>
<td>660</td>
<td>985</td>
<td>37</td>
</tr>
<tr>
<td>CO2 scavengers/emiters</td>
<td>81</td>
<td>108</td>
<td>156</td>
<td>6</td>
</tr>
<tr>
<td>Ethylene scavengers</td>
<td>30</td>
<td>57</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td>Moisture scavengers</td>
<td>190</td>
<td>287</td>
<td>405</td>
<td>16</td>
</tr>
<tr>
<td>Ethanol emitters</td>
<td>21</td>
<td>37</td>
<td>65</td>
<td>2</td>
</tr>
<tr>
<td>Flavour/adsorbers</td>
<td>28</td>
<td>47</td>
<td>70</td>
<td>3</td>
</tr>
<tr>
<td>Antioxidants</td>
<td>3</td>
<td>7</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>Self-venting</td>
<td>195</td>
<td>280</td>
<td>550</td>
<td>16</td>
</tr>
<tr>
<td>Susceptor laminates</td>
<td>80</td>
<td>165</td>
<td>265</td>
<td>9</td>
</tr>
<tr>
<td>Temperature control</td>
<td>15</td>
<td>38</td>
<td>100</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>1064</td>
<td>1786</td>
<td>2556</td>
<td>100</td>
</tr>
</tbody>
</table>
and are excluded from the Directive 2002/72/EC. These materials have to comply with the national legislation which is present in some countries, e.g. Germany and The Netherlands. Under the principle of mutual recognition, other countries should accept food contact materials produced in these countries provided such country has no specific regulation in the relevant fields (Fig. 4).

For the active/intelligent component in active and intelligent packaging, there is no specific Directive or regulation at this moment and they have to comply with the Framework Regulation (EC) 1935/2004. Important articles are Articles 3 and 4. Article 3 is applicable to the packaging of the active or intelligent components. The active and intelligent components are subject to Article 4.

Article 3 of the Framework Regulation states that food contact materials shall not transfer constituents to food in quantities which could:

- endanger human health;
- bring about an unacceptable change in the composition;
- bring about deterioration in organoleptic characteristics thereof.

Furthermore, labelling, advertising and presentation of the material or article should not mislead the consumer.

Article 4 of the Framework Regulation describes special requirements for active and intelligent materials in which the main issues are:

- active materials may bring about changes in the composition or organoleptic characteristics of food on the condition that the changes comply with the community or national provisions applicable to food;
- substances that are released from active packagings shall be authorised and used in accordance with the relevant community provisions applicable to food;
- active materials shall not bring about changes in the composition or organoleptic characteristics of the food, for instance by masking the spoilage of food, which could mislead the consumer;
- intelligent materials shall not give information about the condition of the food which could mislead the consumer;
- adequate labelling to allow identification of non-edible parts;
- adequate labelling to indicate that the materials are active and/or intelligent.

Finally, Article 15 of the Framework Regulation states that the consumer and the food packer must be informed on how to use the active and intelligent materials and articles safely and appropriate.

Draft regulation on active and intelligent packaging materials

In addition to the Framework Regulation, there is a specific Regulation in preparation for the active and intelligent packaging. However, it should be noted that this Regulation is under discussion at the moment and the items described below are based on our personal view based on literature (Actipak, 2001; van Dongen, de Jong, & Rijk, 2007, chap. 9; van Dongen & de Kruijf, 2007, chap. 17).

One of the major issues in this draft Regulation is that the active and intelligent substances require authorisation.
For authorisation, a petition should be prepared of the active/intelligent substance in a similar way as is required for plastics. A guidance document to compile the petition dossier is in preparation.

Other items that will likely be included in the draft Regulation are:

- the released substance is excluded from the overall migration;
- food shall comply with food regulation;
- the active/intelligent packaging should be suitable and effective for the intended purpose;
- materials that may be mistaken as a part of the food (e.g. loose sachets) must be labelled using the symbol for the non-edible part (Fig. 5);
- the declaration of compliance and supporting documentation.

Compliance testing

Directives (82/711/EEC or 85/572/EEC) set requirements for testing the migration from food contact materials. Not all active and intelligent packaging systems can be tested according to these Directives. Distinction should be made between systems inside or outside the primary packaging. Systems outside the packaging need in general no migration testing as there will be a “functional barrier” which reduces the potential migration significantly. In addition, most intelligent systems attached on the outside of the packaged food concerns foods stored for a relatively short period at lower temperatures. The risk of exceeding a lag-time in diffusion is negligible. Nevertheless, there should be a judgement on a case by case basis.

Active and some intelligent systems are always in “direct” contact with food. If the active or intelligent ingredient is incorporated in the plastic film, then such a material shall comply with the conventional rules laid down in the EU Directives. Both positive list and migration behaviour should be in compliance because the way of contact as well as the surface-to-volume ratio is equal to the normal packaging materials.

In other cases, the active or intelligent system is inserted in the primary packaging in the form of a box, sachet, label or sheet, containing the typical active ingredients. Generally, the ratio of food to contact area is large and the contact is not very intense. To establish the requirements of such systems, the type and physical state of the food in contact with the systems should be taken into account. The system may be in contact with dry foods, e.g. coffee, bakery ware, fruits or with liquid foods, e.g. beer, beverages, or with (semi) solid foods, e.g. meat, fish, nuts. In case of dry foods, specific migration of substances should be measured directly in the food, as there are no tests prescribed with simulants. Contact with liquid foods should be tested according to the Directives 82/711/EEC and 85/572/EEC. In these cases, there are no reasons to deviate from the conventional rules. Only in case of (semi) solid foods, there may be problems that need special requirements and testing protocols. New dedicated test protocols should be used for this purpose described by Actipak (2001) and Lopez-Cervantes, Sanchez-Machado, Pastorelli, Rijk, and Paseiro-Losada (2003).

An example is an oxygen scavenger containing iron used for a meat product. Using the conventional test according to Directives 82/711/EEC and 85/572/EEC for the determination of the overall or specific migration, the oxygen scavenger should be immersed in water or 3% acetic acid and stored for the prescribed time—temperature conditions. However, this is an unrealistic situation in case the oxygen scavenger is used for a meat product. For this purpose, a dedicated test is developed to perform an overall migration with filter paper soaked with simulant (Fig. 2). In this test, samples are wrapped with the filter paper which is saturated with simulant and placed between glass plates. To simulate the possible weight of food a total weight of 70 g (including the glass plate of 20 g) is placed on top of the package (see Fig. 6). The whole package including weight is placed in the oven and stored for the correct time/temperature conditions. After the storage period the filter paper are extracted with the food simulant to determine the overall or specific migration.

Comparison of the dedicated test with the conventional test according to Directives 82/711/EEC and 85/572/EEC showed much lower and more realistic overall migration for the dedicated test compared to the conventional test.

In conclusion, active and intelligent packaging systems should comply with the Framework Regulation (EC) 1935/2004. Main requirements in this Framework Regulation are that active materials may bring about changes in the composition or organoleptic characteristics of food on

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**Fig. 5.** Symbol for ‘non-edible’ parts in FCM labelling.

**Fig. 6.** Layout for dedicated migration test for oxygen absorbing label.
the condition that comply with the Community provisions applicable to food. Substances are allowed to be released provided that the food is in accordance with the Food Law. Furthermore, active materials and intelligent packaging should not mislead the consumer for instance by masking the spoilage of food or by giving incorrect information. Finally, adequate labelling is needed. A specific Regulation on active and intelligent packaging is in preparation which will deal with the authorisation of the active and intelligent substances.

Compliance should be tested by following the test protocols described in 82/711/EEC and 85/572/EEC. However, the test protocols may in some cases, such as non-intended contact or semi-solid food, exaggerate the migration and dedicated tests need to be applied.

References


Gontard, N. Tailor made food packaging concept. In: IUFoST, 13th World Congress of Food Science and Technology, Food is Life, 17–21 September 2006, Nantes, France.


Risk—benefit health assessment of food — Food fortification and nitrate in vegetables

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Food authorities should base their decisions on both risk assessment and benefit assessment in relation to foods, food constituents or nutrients when risk—benefit assessment can be carried out. Benefit assessment should mirror the well-established steps of risk assessment. However, methods for their comparison, weighing risks against benefits, have as yet to be developed. The general framework currently available in risk—benefit health assessment of food is described and illustrated with two examples: food fortification with vitamins and minerals, and the consumption of vegetables in relation to their nitrate content.

Introduction

While food must be safe to eat, some foods or food components have the potential to be beneficial and/or adverse to health depending on the population, the amount ingested and the inherent properties of the food/food component. Assessing only the risks or the benefits may provide an unbalanced picture in cases where both aspects coexist. Hence, there is a need for suitable methods to perform risk—benefit assessment and help risk managers to take regulatory and practical actions.

The need for a harmonised science-based methodology for Risk—Benefit Assessment led the European Food Safety Authority (EFSA) to organise a Colloquium on Risk Benefit Analysis of Foods — methods and approaches in July 2006 (EFSA, 2007). This led EFSA’s Scientific Committee to create a working group of experts to prepare a guidance document for risk—benefit assessment of food in relation to human health. This topic was also presented and discussed during the Scientific Forum celebrating the 5th anniversary of EFSA, held on 19 and 20 November 2007 in Brussels. The present paper summarises the contents of these presentations as well as the main points arising from the discussion with the audience on the general approach used in risk—benefit assessment. Finally, two examples illustrate the approach: food fortification with vitamins and minerals, and nitrate in vegetables.

Risk assessment, risk communication and risk management

Risk analysis is a well-established, internationally recognised process, comprised of three distinct elements: risk assessment, risk management and risk communication. Risk assessment is a scientific process, performed independently from risk management, and composed of four steps (hazard identification, hazard characterisation, exposure assessment and risk characterisation) (Renwick et al., 2003). For thresholded toxicants, markers for the thresholds such as No Observed Adverse Effect Level (NOAEL), Benchmark Dose (BMD) or Benchmark Dose Lower Limit (BMDL) (expressed as a dose per kg body

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doi:10.1016/j.tifs.2008.07.005
weight per day) are identified and used to calculate health-based guidance levels or reference values by applying uncertainty factors. For nutrients, the Adequate Intake (AI), Reference Intake (RI), Population Reference Intake (PRI), Upper Limit (UL) are derived. However, the terminology for these reference values is still not yet harmonised (EFSA, 2006). For food additives, pesticides and environmental contaminants the terminology differs i.e. Acceptable Daily Intake (ADI), Tolerable Daily Intake (TDI).

‘Risk management’ means the process, distinct from risk assessment, of weighing policy alternatives in consultation with interested parties, considering risk assessment and other legitimate factors, and, if need be, selecting appropriate prevention and control options (EC, 2002).

‘Risk communication’ means the interactive exchange of information and opinions throughout the risk analysis process as regards hazards and risks, risk-related factors and risk perceptions, among risk assessors, risk managers, consumers, feed and food businesses, the academic community and other interested parties, including the explanation of risk assessment findings and the basis of risk management decisions (EC, 2002).

However, an interactive and reiterative process is needed to ensure that (1) the outcome of the risk assessment is helpful to risk managers, (2) risk communication adequately describes the distinct considerations involved in risk assessment and risk management.

Benefit assessment

The benefit assessment approach proposed by EFSA is a mirror of the above risk assessment paradigm and therefore composed of the four following steps: positive health effect identification, positive health effect characterisation, exposure assessment, and benefit characterisation. “Risk” is understood to be the probability of an adverse effect in an organism, system, or (sub-) population in reaction to exposure to an agent (IPCS, 2004), and “benefit”, the probability of a reduction of the severity of an adverse health effect and/or the probability of an increase of the magnitude of a positive (beneficial) health effect in a group of individuals under defined conditions of exposure (risk—benefit assessment working group of the EFSA Scientific Committee; opinion in preparation).

Risk—benefit assessment

Risk assessment and benefit assessment should be conducted in parallel; they involve different types of data, sources of information, and in some cases may be relevant to different population subgroups. The outcomes of both assessments, as well as their comparison should be provided to the risk manager and specify the assumptions and uncertainties related to the assessments, i.e. input data, extrapolation of animal data to humans, mathematical model selection. The weighing of the benefits against the risks will need to take into account the timeframe for the effects to become apparent and their severity/magnitude.

The decision to undertake a risk—benefit assessment should be made on a case-by-case basis, taking account of the resources and data available, and the potential application and value of the outcome. From the outset, the problem formulation step, involving the risk assessor and the risk manager should involve an iterative exchange as it will determine whether a risk—benefit assessment is needed, what endpoints should be considered, and how the assessment should be conducted (qualitative, semi-quantitative or quantitative).

An intake range within which the balance risk—benefit is acceptable should be identified, especially when intake levels associated with risks and benefits are close. In contrast, when the outcome of the safety assessment shows no appreciable health risk at anticipated dietary exposure, then a risk—benefit assessment is not required. Indeed, in such circumstances, referring to risk—benefit assessment could lead to a misperception that tangible risks for some subgroups could arise.

The following potential cases for risk—benefit assessment were highlighted during the presentations:

- A single compound or food component has the potential for both adverse and beneficial effects in the same population, e.g. selenium.
- A single compound or food component has the potential for adverse effects in one population group and for benefit in another population group, e.g. folic acid.
- The same food contains substances which may cause adverse effects together with substances which may have beneficial effects in the same population group, e.g. the benefit of breast feeding (human milk) vs. environmental pollutants in milk (e.g. persistent organic pollutants).
- The same food contains substances which may cause adverse effects together with substances which may have beneficial effects. The balance risk—benefit can shift towards benefit or risk depending on the subgroup of the population, e.g. the potential benefit of eating fish in adults/the potential risk of neurotoxicity in children and neonates in relation to methylmercury and persistent organic pollutants present in fish.

Existing and future approaches for risk—benefit assessment are also under review (EFSA Working Group on risk—benefit assessment). In addition, several research projects (Beneris, Qalibra, BRAFO1), funded by the Research Directorate General of the European Commission, are also developing methods to compare risk and benefit in a quantitative manner by means of health-related quality-of-life indices, e.g. quality-adjusted life years

(QALYs) and disability-adjusted life years (DALYs) (Murrey, 1994).

The example of nutrients can illustrate the general approach for risk-benefit assessment:

Hazard/positive effect identification

When looking at an essential nutrient, potential adverse effects can occur through: (1) an excessive intake combined with the potential toxicity of the nutrient; (2) inadequate or insufficient intake of the nutrient (independent of the potential toxicity of the nutrient). In the latter case, the positive effect, i.e. risk reduction in nutrient deficiency will solely result from an increase in exposure.

Hazard/positive effect characterisation

A dose–response relationship, with a specific U-shape for essential nutrients, can be determined for each critical effect selected during the first step of the assessment. The optimal range of intake can be identified by combining two dose–response curves describing (1) the risks due to deficiency (absence of benefit) and (2) the risks due to the toxicity of the nutrient. It does not allow the weighing of the severity of the effects but provides a way to determine advisable ranges of intake.

Exposure assessment

Exposure assessment should be carried out for different population groups, estimating the intake of the users vs. the intake of the general population. It is recommended to calculate the respective prevalence of subjects with an exposure above and below the health based guidance levels (for nutrients UL, ARI, PTI). The availability and the quality of the exposure data remain limiting factors, especially for certain subgroups of the population such as children, infants and neonates.

Risk–benefit characterisation

The intake distribution for sensitive subpopulations can be integrated with the dose–response curves. This allows to quantify the fraction of the population that would potentially incur either beneficial effects or a potential increase in the risk of an adverse effect in relation to intake. When communicating the outcome of such risk–benefit assessment, the public health consequences of exceeding the reference values should be described.

Ideally, the output of a risk–benefit assessment should provide risk managers both the outcomes of the risk and the benefit assessment together with a conclusion weighing of risks vs. benefit. Such an advice should only be provided on a case-by-case basis, if the request was voiced by the risk manager during the problem formulation step and included in the question sent to the risk–benefit assessor. Additional information should also be provided regarding data quality and the assumptions and uncertainties of the assessment so that the conclusions sent to the risk manager are clearly formulated in a transparent manner.

Risks and benefits of food fortification

Risk–benefit assessment can become more complex when looking at different subgroups of the population, as is the case for some micronutrients such as vitamins and essential minerals e.g. vitamin A, folate and calcium. The risks and benefits for different subgroups vary respectively according to the variability in biological responses to hazards and positive health effects.

The ideal situation would be to generate a dose–response relationship curve for each risk and benefit, and for each population group. These curves allow the establishment of average requirements and recommended nutrient intake on the nutrition side, and of tolerable upper intake levels on the toxicology side. These curves may overlap either for the same subgroup or for different subgroups. Exceeding the upper level does not give any indication of risks at higher intakes, as it is not a threshold for toxicity; therefore occasional excursions above the upper level are unlikely to be harmful but a prolonged intake above this upper level is undesirable.

Individuals who do not obtain the micronutrients they need from their normal diet will benefit from additional exposure but in some instances this might represent a potential risk for some individuals who already obtain sufficient or more micronutrients than they need from their normal diet, and for subgroups susceptible to micronutrient toxicity.

Calcium fortification

Calcium is required for the formation of bones and teeth, the regulation of muscle contraction and blood clotting, and can be obtained from e.g. dairy foods, green leafy vegetables, soya beans, nuts and small fishes. The Low Reference Nutritional Intake (LRNI) for calcium is 400 mg/day for adults and the Recommended Nutritional Intake (RNI) is 700 mg/day (COMA, 1991).

A mandatory fortification of white and brown wheat flour has been in place in the UK since 1940s; it is estimated that removing this fortification would increase the percentage of the population with intakes below the LRNI, especially among adolescents.

The high intakes for calcium from food in the UK are around 1500 mg/day for adults, i.e. twice the RNI (Henderson, Gregory, & Swan 2002). One should add to this figure the amount of calcium ingested from water consumption and food supplements; the total high level intake for calcium reaches then 4500 mg/day, which is higher than the upper level of 2500 mg/day established by the EC Scientific Committee for Food (EFSA, 2006). These data include current information on voluntary fortification of foods, such as breakfast cereals, but there is potential for even greater exceedance with additional voluntary fortification with calcium of beverages or other foods.

The health implications of exceeding the upper level for calcium are quite uncertain. Based upon the SCF opinion, it is unlikely that these intakes are harmful in the general...
population, but subgroups at risk including patients with renal failure or taking diuretics could be susceptible to hypercalcaemia.

**Vitamin A fortification**

Vitamin A can be obtained from dairy products, oily fish, liver and fat spread; it has a function in eyesight, as well as in embryonic and foetal development. The LRNI is 300 μg Retinol equivalent (RE)/day for men and 250 μg RE/day for women. The RNI is 700 μg RE/day for men and 600 μg RE/day for women (COMA, 1991).

In the UK, the high level of intake is 10-fold higher than the RNI (Henderson et al., 2002) but this does not necessarily mean there is a risk of adverse effects. The SCF upper level of 3000 μg RE/day excluded post-menopausal women (EFSA, 2006). The UK Expert Group on Vitamins and Minerals (EVM) set a guidance level of 1500 μg RE/day for adults, including post-menopausal women (EVM, 2003). The SCF focussed on hepatotoxicity and birth defects, while the EVM also considered decreased bone density and fracture risks.

Fat spreads that are fortified to replace the vitamin A content of butter, as well as liver, fish liver oil and supplements are the major sources leading to high intakes. High intakes are observed mainly in older people due to consumption of liver and liver products.

The health impact of exceeding the upper level will depend on the subpopulation that is exceeding it. An exposure higher than 3000 μg RE/day implies a potential risk of birth defects if taken during pregnancy, and of hepatotoxicity if exceeded on a prolonged basis. Exceeding 1500 μg RE/day could lead to a decrease of bone density and fracture risks for post-menopausal women, but the critical exposure period is unclear. Reducing vitamin A intake by reducing fortification or high food sources in the UK would raise the percentage of men and women with an intake below the LRNI from presently 5.7 and 7.5% to respectively 7 and 9.2%, creating therefore a nutritional risk, but could reduce the risk for fractures, as nobody would exceed the EVM upper guidance level of 1500 μg RE/day. This demonstrates the complexity of the advice that should be given to risk managers, as the evidence relating to the dose—response relationship for both benefit and harm is often inconclusive.

**Folic acid fortification**

Folate has an essential role in cell division and maintenance, as well as in DNA synthesis and repair; natural sources for folate are broccoli, Brussels sprouts, asparagus, liver, chickpeas, brown rice and some fruits. The LRNI is 0.1 mg/day, the RNI is 0.2 mg/day. Supplementation of 0.4 mg folic acid/day is advised from pre-conception to the first trimester in order to reduce the risk of neural tube defects. For high risk pregnancies, a supplementation up to 5 mg/day may be recommended by medical practitioners (COMA, 1991). Benefits have been claimed related to cardiovascular health and cancer risk reduction but the evidence is still incomplete and insufficient to establish any dose—response relationship. Some foods such as fat spreads, cereals and flour can be fortified with folic acid, this chemical form being, however, different in bioavailability and metabolism from the forms of folate that are found naturally in foods. High level folate intake for UK females is 1.29 mg/day, which is higher than the upper level for folic acid set by the EC SCF and UK EVM at 1.0 mg/day (EFSA, 2006; EVM, 2003).

The health risk associated with exceeding the upper level would be masking of vitamin B12 deficiency, and as a result allows the undetected development of neuropathy. This has been observed at folic acid supplementation higher than 5 mg/day. Older people are most susceptible to this effect. There is uncertainty with respect to the risk of supplemental folic acid between 1 and 5 mg/day (EVM, 2003). From carcinogenicity studies in animals, there is a biological plausibility of increased risk of cancer at insufficient doses of folic acid, as well as at excessive doses of folic acid; however, the intake range where the cancer risk is the lowest is unclear. A recent study (Cole et al., 2007) suggested that folic acid at high doses could promote pre-neoplastic lesions. The subjects in this study were administered a supplemental folic acid dose of 1 mg/day during a period in which mandatory fortification was introduced and there was no control on voluntary fortification. Therefore, exposure to folic acid in these subjects was above the EU tolerable upper level of 1 mg/day but uncertainty remains over the exact levels.

The current UK policy aims at reducing the incidence of neural tube defects by increasing intakes by average of 80—100 μg/day. The mandatory fortification of bread or flour with folic acid would reduce the number of persons not achieving the RNI to 5% and maintain individuals exceeding the upper levels to the current levels. By controlling voluntary fortification and publishing advice on supplement use, the UK FSA aims to ensure that folic acid reaches those people with intakes within the lowest quintile of consumers, without putting high intake consumers at risk (FSA, 2007).

**Conclusion on fortification**

Micronutrient requirements vary depending on the life stage and the population subgroups considered, therefore intakes that are beneficial for some people could be harmful for some others. There is a need for detailed information on dose—response relationships to characterise both sides of the equation, i.e. the risks and the benefits; unfortunately this information is generally lacking. Whilst fortification could benefit people with an otherwise low intake, it could represent a risk for people who already have a high intake. It is therefore crucial, when assessing risks and benefits, to take the dietary habits of the relevant subgroups into consideration.
Nitrate in vegetables – a risk–benefit assessment example

The WHO recommends 400 g fruit and vegetable per day which approximates to 5 portions/day (WHO, 2003).

There is general consensus that eating vegetable is an important component of a healthy diet if consumed in sufficient amounts, with potential beneficial health effects as demonstrated in observational studies describing the relationship between fruits, vegetables and health. However, concerns related to the presence of *inter alia* anti-nutrients (e.g. lectins, glucosinolates), nitrate, allergens, mycotoxins, contaminants and pesticide residues in vegetables have been raised. Leafy vegetables, in particular (e.g. lettuce, rucola, spinach) contain naturally high levels of nitrate (NO$_3^-$) which together with its metabolites (nitrite (NO$_2^-$) and nitric oxide (NO)) have not only physiological and potential beneficial roles in the body, but also potential for adverse effects. Both aspects need to be balanced in relation to the consumption of vegetables and are discussed below, as part of the conclusions of the EFSA Scientific Panel on Contaminants in the Food chain (CONTAM) (EFSA, 2008).

Toxicity and potential beneficial effects of nitrate and its metabolites

After oral intake of exogenous nitrate, around 5–20% of the dose is converted to nitrite at the back of the tongue by nitrate reductase. There is also endogenous pathway representing approximately 1 mg/kg b.w. per day for a 70 kg adult (Archer, 2002; Lundberg, Weitzberg, & Gladwin, 2008). In healthy adults 5–7% of the total absorbed nitrate intake, goes through an entero-salivary recirculation pathway and is then concentrated approximately 10 times in the saliva, metabolised at the back of the tongue by commensal bacteria to nitrite resulting in approximately 20% being swallowed as nitrite. Evidence from the excretory patterns in the kidneys, gut, and saliva confirms that nitrite and nitrate are conserved through recycling in the body, as well as being endogenously produced.

Nitrate can be further reduced to nitric oxide which has a range of well known physiological functions in the body (Lundberg *et al.*, 2008).

The potential adverse health effects of nitrate are in fact mediated via its metabolite nitrite (EFSA, 2008) and several contributing factors such as intestinal infection, inflammation can lead to the formation of methaemoglobin (metHb), a ferric form of oxidised haemoglobin that is unable to bind oxygen. Raised levels of metHb in infants under 4–6 months can cause the blue baby syndrome (FAO/WHO, 2003a, b). Another toxic effect of nitrate, mediated by nitrite and ultimately nitric oxide, is the potential for reaction with amino substrates to form N-nitroso compounds, which in turn may form nitrosamines. Nitrosamines have been shown to cause cancer in experimental studies, particularly gastric tumours (Mensinga, Speijers, & Meulenbelt, 2003). However, a number of studies have indicated that when nitrate is consumed in vegetables where other bioactive substances such as antioxidants are present, nitrosamine formation can be reduced by up to half (Brambilla & Martelli, 2007).

Regulated nitrate levels

In response to the potential for adverse health effects, maximum nitrate levels for vegetables are set in Regulation (EC) No 1881/2006 and range from 2000 mg/kg frozen spinach to 4500 mg/kg fresh lettuce (excluding iceberg-type lettuce; grown under cover and harvested from 1st October to 31st March). These reflect a trend for higher levels of nitrate in vegetables during winter, as well as in vegetables grown under glass/plastic cover. Thus light intensity is an important determinant of the nitrate level in vegetables. Leafy vegetables that draw nutrients from the roots to the leaves tend to accumulate more nitrate in their leaves compared to seeds, cereals and other types of vegetables.

Nitrate intake

Throughout the diet, nitrate is mainly found in vegetables and fruit$^2$, animal based products i.e. cured meat, water and beer. For the UK and France, estimates of total daily dietary intake of nitrate are about 91 and 141 mg/person/day with 52 and 75% from vegetables and fruit, respectively. Dietary nitrite intake is much lower with respective values of 1.5 and 2 mg/person/day for the UK and France. However, the total nitrite exposure is approximately 7.3 and 11.3 mg/person/day in the UK and France which takes into account endogenous conversion from nitrate.

Nitrate in vegetables

Nitrate levels for 59 individual vegetables resulting in 42000 analytical results (covering the years 2000–2007) were submitted to EFSA by 20 European countries and grouped in nine categories. Nitrate median levels ranged from 1 mg/kg in some vegetables (peas) up to 4800 mg/kg for ru cola with the highest value reported for a lettuce (oak leaf lettuce) sample at 19,925 mg/kg which is nearly 20 g/kg. Such high levels of accumulated nitrate could become a health concern for a faddy eater with a poor diet, additionally taking into account background nitrate exposure from other sources. Of the nine categories, herbs, leafy vegetables and stem vegetables have the highest nitrate values, and within leafy vegetables ru cola (rocket) is at the highest with a median concentration of 4800 mg/kg (EFSA, 2008). It should be noted that nitrate levels are reduced during processing and cooking (Roszczenko, Rogalska, Potapczuk, & Kleczyńska 2001).

Consumer intake

In assessing intake, there is no such thing as a standard European consumer. In this assessment, a range of dietary patterns and locations were taken into consideration.

$^2$ However, the nitrate content of fruit is relatively low (10 mg/kg).
throughout the EU. The GEMS/Food Consumption Cluster Diets database (WHO, 2006) was used as well as consumption information supplied by 11 European Member States and Norway. The figures viewed overall gave a highest (97.5th percentile) daily intake of “most vegetables” just below 400 g/day. This amount, taking a conservative approach, is similar to the WHO recommendation that the daily intake of fruit and vegetables should be about 400 g/day. For the risk assessment, for the base case, it was assumed that all of the 400 g were consumed in the form of vegetables. In addition high consumer intakes also have to be considered, such as the potato intake in Ireland (771 g/day). The highest reported level of leafy vegetable (lettuce) consumption in EU is from Spain, approximately 133 g/day, which is about 1/3 of the 400 g base case vegetable total intake.

For nitrate intake the main driver is not the amount of vegetables eaten, but the type of vegetable and nitrate level which is the determining factor together with variables such as the production type, light, fertiliser use, and temperature.

Nitrate exposure and the Acceptable Daily Intake

No new toxicological data were available since the latest evaluation by the Joint FAO/WHO Expert Committee on Food Additives (JECFA); the ADI of 3.7 mg/kg/day for nitrate as established by JECFA (FAO/WHO, 2003a, b) was retained by EFSA (EFSA, 2008). For a 60 kg adult, the ADI would correspond to a total intake of 222 mg nitrate/day. The exposure from a balanced diet of a 400 g mixed vegetables/day would give an intake of 157 mg NO_3/day which is about 70% of the ADI. Even allowing for other background sources of nitrate (35–44 mg/person) the total combined intake remains below the ADI. Another intake example, where 133 g spinach (higher in nitrate than mixed vegetables) was combined with mixed vegetables remains below the ADI. Some intake scenarios could theoretically exceed the ADI, although a number of mitigating factors need to be taken into account. These include: (1) the use of conservative modelling where much of the 400 g assumed as vegetable consumption would in fact be in the form of fruit with low nitrate content (10 mg/kg), (2) processing such as peeling and cooking of some vegetables which can significantly reduce nitrate levels. Some studies have shown reduction of nitrate levels (16–79%) when vegetables are cooked in water particularly for peas, cabbage, beans, carrots, potatoes and spinach, endives and celery during cooking (Dejonckheere, Steurbaut, Drieghe, Verstraeten, & Braeckman, 1994; EFSA, 2008; Roszczenko et al., 2001). However, these figures are very variable and the CONTAM panel pointed out the need for further data with regards to the influence of productions, storage and processing on nitrate and nitrite concentrations (EFSA, 2008).

Balancing the risk and benefits of nitrate

In the case of non-communicable diseases, cardiovascular disease affecting approximately half of the population and cancer affecting 1/3—1/4 of the population top the list. Recent reviews are in agreement, that eating five portions a day of fruit and vegetables, roughly equivalent to 400 g, can have a beneficial impact on individual susceptibility to cardiovascular disease, cancer, obesity and type 2 diabetes (WHO, 2005).

In considering nitrate and vegetables, the pros and cons for health have to be considered in the context of the developing understanding of the physiological roles played by this substance and its metabolites. On the benefit side, exogenous nitrate may complement endogenous production, nitrate, as nitric oxide in an acid pH contributes to the host defence system to kill enteropathogens and nitric oxide is a signalling molecule that plays a role in inflammation and vaso-regulation (Lundberg et al., 2008). Recently, high nitrate levels of dietary nitrate (1.45 g) were administered to healthy adults as beet root juice and plasma nitrates correlated with a reduction in blood pressure and markers of inflammation after an ischemic insult on the forearm (Webb et al., 2008).

Although food is required to be safe, there is no such thing as zero risk and there can be risk factors associated with the consumption of some vegetables containing naturally compounds with potential deleterious effects e.g. anti-nutrients or allergens. On the beneficial side, with regard to vegetable consumption there is general agreement that their content of macronutrients (e.g. fibre), micronutrients (e.g. vitamins), and bioactive substances results in an overall health benefit (WHO, 2005).

Conclusion on nitrate

A minority of Europeans eat 400 g of vegetables/day, a day as at least half of this recommended intake will typically be in the form of fruit for which there is a general preference. As the nitrate level is generally low in fruit (around 10 mg/kg) the majority of the population have a nitrate intake below the ADI. At these nitrate levels 400 g of vegetables or for most people fruit and vegetables will have a positive health impact. However, there are consumers with a high leafy vegetable intake, increasingly of raw leaves, who could conceivably exceed the ADI. Whilst exceeding the ADI does not necessarily constitute a risk to health, it is undesirable and for the protection of high level consumers the nitrate levels need to be kept as low as reasonable possible, by ensuring that good agricultural practice is applied. Overall, the CONTAM panel concluded that the estimated exposures to nitrate from vegetables are unlikely to result in appreciable health risks, therefore the recognised beneficial effects of consumption of vegetables prevail (EFSA, 2008).

Overall conclusion

The human health risk assessment of food constituents is an internationally agreed and well-established process. The recognition that some foods/food substances have the potential to exert both health risks and health benefits requires
to an expansion of the basic risk assessment process to allow a scientific evaluation of the benefits as well as the risks. Such risk—benefit assessment should be decided on a case-by-case basis, particularly when the levels of intake associated with risks and benefits are close. The outcomes of both the risk and the benefit assessments, the comparison between risks and benefits, and a detailed description of the assumptions made and uncertainties linked to the assessments should be provided to the risk managers to take regulatory and practical actions.

EFSA and several EU-funded research projects are currently cooperating in order to develop a harmonised science-based methodology for risk—benefit assessment, and identify suitable ‘common currencies’ that would allow a quantitative weighing of risks and benefits.

References