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Timely awareness and prevention of emerging chemical and biochemical risks in foods: proposal for a strategy based on experience with recent cases

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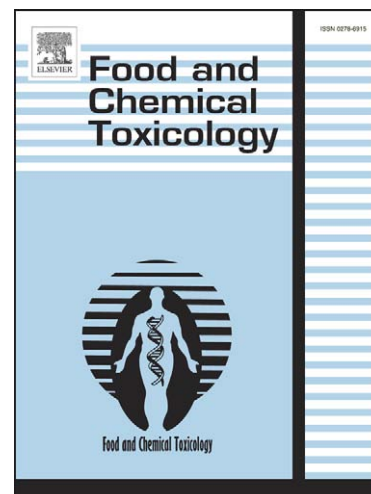
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**TIMELY AWARENESS AND PREVENTION OF EMERGING CHEMICAL AND BIOCHEMICAL RISKS IN FOODS: PROPOSAL FOR A STRATEGY BASED ON EXPERIENCE WITH RECENT CASES.**

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**ABBREVIATIONS**

ADI, acceptable daily intake; APHIS, Animal and Plant Health Inspection Service; BEN, Balkan endemic nephropathy; CAP, chloramphenicol; CDC, Center for Disease Control and Prevention; CHN, Chinese herb nephropathy; DNA, deoxyribonucleic acid; EC, European Community; Cry protein, protein from crystalline inclusions of spores formed by *Bacillus thuringiensis*; EFSA, European Food Safety Authority; EMEA, European Agency for the Registration of Medicinal Products; EMRISK, EFSA-sponsored project on forming a global system for identifying food-related emerging risks; EPA, Environmental Protection Agency; EU, European Union; FAO, Food and Agriculture Organization of the United Nations; FDA, Food and Drug Administration; FIFRA, Federal Insecticide Fungicide and Rodenticide Act; GC, gas chromatography; GM, genetically modified; GMO, GM organism; HACCP, hazard analysis critical control points; ISO, International Standards Organization; JECFA, Joint FAO/WHO Expert Committee on Food Additives; MPP+, 1-methyl-4-phenylpyridinium; MPTP, N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MRL, maximum residue level; MRPL, minimally required performance level; MS, mass spectrometry; PeriApt, EU-project on pan-European proactive identification of emerging risks in the field of food production; SAP, Scientific Advisory Panel; TGEV, Transmissible Gastroenteritis Virus; TLC, thin-layer chromatography; UK, United Kingdom; US, United States; USDA, US Department of Agriculture; WHO, World Health Organization

**ABSTRACT**

A number of recent food safety incidents have involved chemical substances, whilst various activities aim at the early identification of emerging chemical risks. This review considers recent cases of chemical and biochemical risks, as a basis for recommendations for awareness and prevention of similar risks at an early stage. These cases include examples of unapproved genetically modified food crops, intoxications with botanical products containing unintentionally admixed toxic herbs, residues of unapproved antibiotics and contaminants in farmed aquaculture species such as shrimp and salmon; and adverse effects of chemical and biological pesticides of natural origin. Besides case-specific recommendations for mitigation of future incidents of the same nature, general inferences and recommendations are made. It is recommended, for example, to establish databases for contaminants potentially present within products. Pro-active reconnaissance can facilitate the identification of products potentially contaminated with hazardous substances. In international trade, prevention and early identification of hazards are aided by management systems for product quality and safety, rigorous legislation, and inspections of consignments destined for export. Cooperation with the private sector and foreign authorities may be required to achieve these goals. Whilst food and feed safety are viewed from the European perspective, the outcomes also apply to other regions.

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## 1. Introduction

Within the area of food safety, consumers and producers may face various hazards that are of widely different nature, including chemical, microbiological and physical agents. Microbiological agents are commonly associated with comparatively acute symptoms, which may appear within days after consumption of a tainted food. However, for chemicals, both acute and chronic toxicities are known. In the latter case, the effects may only reveal themselves after a prolonged period of exposure to low-level contamination. Therefore, chronic toxicity, as well as carcinogenicity, teratogenicity etc. also need to be considered if the harmful effects of a chemical are assessed. The harm caused by a single high dose of a chemical may not equal prolonged exposure to lower doses.

In the last decade, a number of chemical risks have led to food safety incidents in the European Union (EU) and elsewhere. Some examples of these incidents include:

- Dioxins in vegetable oil admixed with industrial oil and used in poultry feed;
- Non-authorized antibiotics, such as chloramphenicol (CAP) and nitrofurans used in aquaculture, in imported shrimps and poultry products;
- Acrylamide, a compound considered to be a carcinogen, in heated products, such as French fries and cereals;
- Industrial dyes, in particular Sudan red, in spices and oils; and
- Dioxins in cow's milk due to their occurrence in the animal feed derived from by-products of potato processing containing dioxin-contaminated kaolinite clay.

It were food safety incidents like these that led to the establishment of the European Food Safety Authority (EFSA), for independent risk assessment and risk communication regarding food safety issues in the EU. The requirement for the establishment of this authority at the European level, as well as of national food

authorities in each of the EU member states has been laid down in the General Food Law, Regulation 178/2002/EC (this EU regulation and others mentioned in the following sections can be retrieved from the Eur-Lex website at <http://europa.eu.int/eur-lex>). These steps were considered to facilitate food risk analysis, since previously these tasks were carried out by multiple institutions in a less coordinated manner.

These incidents also illustrate the need for awareness of emerging risks at an early stage. Early awareness of emerging risks could in retrospect have enabled timely measures aimed at preventing these potential risks from developing into real ones, reducing the negative impact on the economy and possibly health. In fact, it is within EFSA's remit to identify emerging issues and to inform the EU about them.

The definition of "emerging" used here applies to the following categories of risk:

- Risks that have previously occurred and subsequently subsided, but that are now re-occurring. These are "re-emerging" risks.
- Risks that were previously unknown, but that we have become aware of thanks to the progress in science and knowledge
- Risks that were previously not present within foods in general, or in a particular type of food, and that have started to emerge in these products. This emergence might be due, for example, to a change in the dispersion of the risk or the production method of the food.
- Risks related to compounds that were previously present in foods, but to which the exposure of consumers has increased to such extent that they have become a risk (excess of the safety threshold)

Perhaps "hazard" would be a better description of an emerging risk in an early phase of development instead of "risk" as mentioned above. For both terms, official definitions used by international bodies like the Codex alimentarius (1999; 2004) are in use. According to these definitions, a chemical hazard, for example, is a chemical agent with the potential to cause an adverse health effect. The risk associated with the hazard is the function of the probability of the adverse health effect and the severity of that effect, as a consequence of the said hazard. In other words, a hazard may be a toxic compound (for example, a food contaminant), while the risk is the likelihood and the severity of toxic effects following exposure to this compound (for example, by consumption of contaminated food).

Various emerging food safety hazards, including microbiological agents as well as mycotoxins formed by moulds growing on food and feed crops, are discussed elsewhere in this special issue. This article focuses on emerging food risks to the health of consumers caused by compounds of a chemical and biochemical nature, except for the mycotoxins mentioned above. Throughout the article, these risks will be referred to as "chemical risks."

The purpose of this review is to gain insight in how to enable the awareness and prevention of emerging risks in early stage of their development, actually before they become real risks to the consumer. The EU is where the scene for these emerging risks is centered. However, the case studies featured in this report pertain to products that are to a substantial degree imported from non-EU nations into the EU. These case studies also focus on chemical risks linked to botanical products and genetically

modified (GM) crops, since they are considered characteristic for low- and high-input agriculture, respectively. Finally, conclusions are drawn and recommendations made based on the outcomes of the case studies.

## **2. Case studies**

There are a number of parallel activities within the EU that aim to support EFSA in its task to establish a system and procedure to identify emerging risks in an early stage. Examples of this are the EFSA-sponsored PeriApt (VWA, 2005) and EMRISK projects (EFSA, 2006a), as reviewed by Kleter and Marvin (2008) and by Marvin et al. (2008). This followed a series of incidents affecting food safety in the EU. An approach taken by some of the projects and also within the current work is to carry out a retrospective analysis of these incidents. Various case studies have been chosen for this review on emerging chemical risks, which are treated in more detail below. These studies include a retrospective analysis of food incidents involving chemical risks, including the intoxication of consumers by botanical products, the admixture of non-authorized GM crops with commodities, and the presence of antibiotics in imported shrimps and contaminants in farmed salmon. Despite the fact that all the cases studied occurred in the past, they may be valuable in gaining insight in how to prevent similar cases in the future. Another case study treats pesticides of natural origin, with an example of the media attention surrounding the potentially adverse effects of one of these pesticides, rotenone. Although not a food incident, the past incidents with synthetic pesticides and the recent trend towards natural ones convey contemporary relevance to this case study. The case studies on antibiotics in shrimp, on contaminants in salmon, and on pesticides of natural origin are partially based on more detailed annexes prepared by the same authors for two Dutch project reports on emerging risks (Kleter et al., 2006a,b). Finally, conclusions and recommendations have been formulated based on the outcomes of the studies.

### **2.1. Unauthorized GM products**

Over the last decade, the large-scale cultivation of GM crops has increased rapidly world wide, particularly in a number of countries outside the EU. Before such crops are allowed to be used for cultivation and for food and feed applications, they have to undergo a regulatory approval procedure, which includes an assessment of their safety. Given that a number of GM crops that are grown outside the EU have not yet been approved inside the EU, the chance that unauthorized GM crops and derived products may be present in imported consignments cannot be completely discounted. Some other GM crops have been developed for non-food applications, including for medicinal and industrial purposes, and commingling of these crops with their conventional or approved GM counterparts that are used as food or feed should be avoided.

Incidents of accidental commingling of non-authorized GM crops with conventional crops have recently occurred (Table 1). The diversity of GM crops is likely to increase in the near future, especially outside the EU in major crop-growing areas. Besides the mainly agronomic traits of the GM crops that are currently on the market, these new crops may have traits that are consumer-oriented, for example “Golden Rice” with increased levels of pro-vitamin A. Non-food applications for GM crops are also envisaged, such as the production of non-food specialty chemicals, for example industrial enzymes and medicinal substances. Not all of these crops may have been notified for approval in the EU. Therefore, the accidental commingling of

unauthorized GM crops with imported commodities is an important issue to be considered.

<< Insert Table 1 around here >>

Commingling may take place in multiple phases of the crop production cycle. During seed production, for example, this may occur if plots of GM crops in the vicinity of those with seed crops can cross-hybridize by pollen flow. Commingling may occur also further down the chain, such as during cultivation of the seed, transport, storage, processing into food and feed. Harvested crops may also contain GM varieties if crop seeds have been sown that were commingled with GM varieties.

The EU is currently preparing legislation on permissible levels of adventitious commingling of seeds with authorized GM varieties, taking into account the thresholds that have already been set for the adventitious presence of such varieties in food and feed. If the adventitious presence exceeds these thresholds, the seed lot must be labeled as GM seed. In more general terms, it should be noted that special isolation distances are required for seed production in the EU and elsewhere, which may facilitate the prevention of cross-hybridization.

#### **2.1.1. Authorization of GM products in the EU**

Before GM products are allowed onto the market in the EU, they must have received regulatory approval by the EU. There are a number of EU regulations covering different aspects of applications for GM products. For example, food and feed applications of GM products are covered by EU Regulation 1829/2003/EC. This regulation stipulates the procedure for the submission and assessment of data provided by applicants (*i.e.* biotech companies producing GM products) that want to bring the GM products to the market.

The applicant has to compile a “dossier” containing information with regard to the genetic modification, characteristics of the host plant, safety data, labeling measures and, if applicable, post market monitoring. This dossier is submitted to a Member State, *i.e.* the “rapporteur”, which relays the dossier to EFSA. EFSA will solicit comments from EU Member States and also ask its Scientific Panel on Genetically Modified Organisms (GMOs), which consists of independent experts on GMO biosafety, to conduct a safety assessment.

The safety assessment of the dossier follows the internationally harmonized consensus approach of the “comparative safety assessment”, which is also known as “substantial equivalence” (Kok and Kuiper, 2003). The assessment focuses on the differences between a GMO and its conventional counterpart, usually by comparing their agronomic, phenotypic, and compositional characteristics. Compositional analysis, for example, commonly includes key nutrients (*e.g.* oil, protein, vitamins) and anti-nutrients (*e.g.* phytic acid) of a given crop. These differences then become the focus of the further safety assessments. Issues that are commonly addressed during the safety assessment are potential toxicity and allergenicity, horizontal gene transfer, nutritional value, and unintended effects. The EFSA Panel on GMOs has published guidance documents on the safety issues to be considered by applicants for the submission of dossiers on GMOs, such as GM crops (EFSA, 2004a).

Besides the scientific safety assessment that GM products have to undergo, EU Regulation 1829/2003/EC also stipulates that these products should be labeled as being GM, which is elaborated further in Regulation 1830/2003/EC. This requirement also pertains to food and feed in which no traces of GM material are detectable, such as purified seed oils. For the enforcement of this labeling requirement, the regulation stipulates that a documentary system be set up, in which each participant in the manufacturing chain (except for retailers' sales) keeps records of the GM products received from suppliers and sold to their customers.

Thresholds have been set for the adventitious presence of GM products in food and feed products, *i.e.* the accidental commingling of GM with other products for which the manufacturer / trader can prove that it tried to keep them separate. This threshold for GM products approved at EU level has been set at 0.9 percent. Another threshold of 0.5 percent has been set for GM products that have received a favorable scientific opinion from the EU regarding safety, but that have not been approved under the former regulatory regime. Moreover, the applicant has to submit a detection method and reference materials of the GM product to the Joint Research Centre of the European Commission, which acts as the Community Reference Laboratory and thus will validate the method. It also has to carry out co-existence measures on the national level of each member state.

Based upon the Panel's opinion, EFSA will advise the European Commission, which will draft a decision and propose this to a regulatory committee (Standing Committee on the Food Chain and Animal Health) and the Council of Ministers before finalizing a decision.

Directive 2001/18/EC covers the environmental release of GM plants, including cultivation (for field trials or commercial purposes), import, and processing. For food and feed use, additional permission under 1829/2003/EC is required (see above). The procedure for the submission of dossiers is largely the same as for 1829/2003/EC, except for the fact that the "rapporteur" Member State initially carries out its own risk assessment before relaying the dossier to the European Commission and EFSA.

An important part of the safety assessment is the environmental risk assessment. One of the environmental risks is the potential out-crossing of the GM crop with wild relatives through pollen flow. Maize, for example, is a crop that has its origins in Latin America and that has become domesticated in Europe. No wild relatives are known to occur in Europe, with which maize can outcross. Other considerations include, among others, the likelihood that the GM crop could become a weed itself or that it would be harmful to non-target organisms. With the importation of a GM crop, the consequences of spilling viable seeds during transport may need to be considered during the safety assessment.

As regards the environmental safety of GM medicines, Directive 2001/18/EC mentions that a safety assessment is not required under this directive, provided that an equivalent assessment is done under the legislation for medicines. Therefore, the environmental safety of the GM component of medicines has also to be considered during their pre-market safety assessment under the medicinal legislation. New human and veterinary medicines, *i.e.* substances that are claimed to mitigate, prevent, or diagnose disease, have to be approved before they are allowed to be marketed in

the EU. A centralized procedure exists for the notification of medicines, somewhat similar to the scientific evaluation of GMOs by EFSA, which are to be evaluated by EMEA, the European Agency for the Registration of Medicinal Products. In addition to that, a de-centralized EU procedure for medicines is in place. A number of medicines that have been notified to EMEA are of GM nature, such as “biologics”, including vaccines and purified proteins produced by GM animal cells.

Usually, the testing of medicines before they enter the market is rigorous and includes preclinical testing (*i.e. in vitro* laboratory assays, *in vivo* animal toxicity testing, etc.) and clinical tests with increasing numbers of human volunteers. Moreover, given that medicines are expected to have physiological effects, including possible side-effects, post-market monitoring is mandatory.

### **2.1.2. Recent examples of commingling**

Two retrospective examples have been chosen, in which incidents involving the commingling of crops used for food with unauthorized GM crops are studied. Whereas both incidents actually occurred in the United States (US), they may still provide valuable insights for the situation in the EU.

#### **2.1.2.1. Starlink™ maize**

The first example of commingling is that of Starlink™ maize, which was a GM crop that had been approved for feed use only and not for food use by the US authorities. Maize is converted into various products for food and feed consumption. The green part with or without kernels can be used as fodder for domestic animals, for example, as forage or silage (after fermentation), usually close to the place of cultivation. Kernels (from harvested cobs) can be processed through wet-milling into starch and maize germ oil for human food consumption, while some by-products, such as maize gluten, can be used for animal feed consumption. Dry-milling products, such as maize grits, are also used for human food purposes, *e.g.* “maizena” or “polenta”. Besides these uses for maize, whole kernels of sweet maize varieties are used as human food, while other specialty types of maize may be used for popcorn.

The reason for the distinction between food and feed use of Starlink™ was that there was still some uncertainty as to whether the transgenic Cry9C protein expressed by this maize might have the potential to become an allergen to human consumers. The Cry9C protein originated from the bacterium *Bacillus thuringiensis*, of which various strains have been used as biological insecticide for decades in agriculture and forestry. These bacteria produce parasporal crystalline inclusions containing insecticidal proteins known as “Cry” proteins, which are active against specific insect species, but which exert no adverse effects on humans or domestic animals.

Some Cry proteins have been introduced into GM crops, conveying resistance towards certain insect pests. For example, the Cry9C protein introduced into Starlink™ maize is active against certain types of lepidopteran pest species (*i.e.* larvae of moths). The protein introduced into Starlink™ is a truncated form containing the active part of the naturally occurring protein. Such an active fragment is also formed in nature in the intestines of insects ingesting the protein.

One amino acid in this Cry9C protein has been mutated in order to increase the resistance of the protein towards degradation in the intestines of the target insect.

This resistance towards gastro-intestinal digestion particularly raised the concern that there might be an increased allergenic potential for this protein. Transgenic proteins are usually tested for their susceptibility to degradation in the *in vitro* model system of simulated gastric fluid containing pepsin in diluted hydrochloric acid. If the protein proves to be resistant towards digestion by pepsin, it may be considered more likely to sustain digestion and become available for uptake in the gastrointestinal tract. In case of such stability, the protein might be able to interact with the mucosal immune system and prime an allergic response. If the protein possesses toxic properties, it might thus cause adverse effects. Testing the susceptibility of newly introduced proteins to pepsin degradation is part of the “weight of evidence” approach recommended by Codex alimentarius for the assessment of potential allergenicity (Codex alimentarius, 2003). Other items considered within this recommended approach are:

- The allergenicity history of the source of the foreign gene
- Similarity between the amino acid sequence of the novel protein with sequences of known allergens, and,
- Screening with sera from patients allergic to a particular allergen in some cases.

The potential allergenicity of the Cry9C was a point of consideration during the evaluations of Starlink™ maize for market approval by the American authorities before the incident occurred. Besides the stability of Cry9C towards pepsin digestion, it has also proved stable towards trypsin digestion and to heat treatment. Other data from an experimental animal study had also been provided. The data showed an allergic serum reaction of Brown Norway rats towards the Cry9C protein. However, the animal model had not been evaluated for the purpose of potential allergenicity and another protein not known to be allergenic also scored positively. The company also submitted data from sera screening of patients allergic to non-GM maize, which did not show altered allergenicity of Starlink™ maize compared to control. This did not concern the allergenicity of Cry9C protein itself but the intrinsic allergenicity of the maize host crop.

These considerations of the Cry9C protein’s stability led the American authorities (Environmental Protection Agency, EPA) to their decision in 1999 to allow the use of the Cry9C protein as “plant-incorporated pesticide” in Starlink™ maize for animal feed purposes only (Bucchini and Goldman, 2002). An exemption was also made for a tolerance threshold for Cry9C in raw agricultural commodities, *i.e.* in maize for feed use only and in edible products derived from animals fed this feed. Based on their low levels of occurrence in GM crops, EPA has also exempted other transgenic proteins introduced into crops and considered to be safe. Previously in 1998, Starlink™ maize had been deregulated by the US Department of Agriculture (USDA), which, together with the EPA approval of Cry9C, enabled commercial cultivation. The company had offered the EPA a plan to track Starlink™ maize and keep it separated from the food chain, by the use of buffer zones by contracted farmers. Meanwhile, the EPA also had asked its Scientific Advisory Panel (SAP) on issues regarding the US Federal Insecticide Fungicide and Rodenticide Act (FIFRA) to assess the safety of Starlink™ maize for food use (Bucchini and Goldman, 2002).

Several years after the introduction of Starlink™ onto the market, presence of traces of this maize in taco shells and other human food products was reported. These data

implied a breach of the conditions under which Starlink™ maize had been approved for marketing, *i.e.* animal feed use only. This finding led to a major recall action of retail food products as well as the buy-back of seeds and harvests that potentially contained Starlink™. Companies pro-actively tried to avoid the sales and purchase of Starlink™-containing maize.

The US authorities also carried out a risk assessment regarding the potential allergies that the exposure of the American consumers to Starlink™ might have caused. As noted above, the FIFRA SAP at that moment was still evaluating food safety data on the Cry9C protein. Following the news concerning the commingling of Starlink™ with maize used for food purposes, FIFRA SAP assessed the likelihood that Cry9C might be allergenic. It concluded that Cry9C had a medium likelihood of being an allergen and that exposure of the US consumers, including Hispanic communities which have a comparatively high level of consumption of maize products, probably had been low (EPA, 2000; EPA, 2001).

Finally, citizens were asked by the US Food and Drug Administration (FDA) to report allergic reactions that might have been related to the consumption of Starlink™ maize. The individuals that thus had identified themselves volunteered to participate in screening of their sera for IgE-antibody binding to Cry9C by the US Center for Disease Control and Prevention (CDC). No sera reaction to Cry9C could thus be established. It should be noted that the Cry9C protein used in this assay was the recombinant counterpart produced by GM bacteria, since purification of the low levels of Cry9C from plants would be cumbersome. Moreover, in one of the subjects who had participated in the CDC trial, a double-blind, placebo-controlled food challenge with Starlink maize failed to confirm allergy towards this maize (Sutton et al., 2003).

From a regulatory point of view, the American authorities revoked the previous approvals of Starlink™ maize. A petition of the company to the American authorities to allow very low levels of Starlink™ in maize destined for food was not completed successfully. The US authorities also started controlling export maize commodities for the presence of Starlink™ before being shipped to foreign ports. Authorities in receiving ports have also included Starlink™ in their detection schemes with examples of reports of Starlink™ being detected in shipments that arrived in Japanese seaports.

The authorities further changed their regulatory policy by only issuing approvals of GM crops for both food and feed uses, but not for either of these uses separately. Whereas Starlink™ maize had not been approved in the EU, the recently adopted EU regulations on GM food and feed, such as Regulation 1829/2003/EC, actually stipulated the policy towards the approval of GM crops for both food and feed but neither separately.

The transgenic Cry protein had been modified such that it had become more stable towards digestion by target insects. Through this modification, it probably also had become more resistant towards digestion by consumers (humans, animals), one factor that might contribute to an increased likelihood of allergenicity. In contrast, Cry proteins from other approved GM crops had been shown to be digested rapidly by

pepsin. This was also one of the main reasons why Starlink™ maize had initially only been approved for feed use and not for food use.

Another issue is the scientific uncertainty that surrounded the question which the FIFRA SAP had to answer about the threshold above which an allergen can sensitize a person to become allergic to the allergen, *i.e.* the minimum intake of an allergenic protein. The panel was unable to reach a conclusion on this issue. Therefore, it could not directly compare this to the exposure data.

### **2.1.2.2. Medicine-producing GM crops**

The second example of commingling is that of GM maize producing medicinal substances. This case is different from Starlink™ in that it concerns an experimental crop of which the potential admixture to other crops was detected in an early stage. The contaminant maize in this case was intended for medicinal purposes and hence not for food or feed purposes. GM crops with medicinal traits that are currently on the market include predominantly bovine pancreas trypsin produced in maize (TrypZean™), and to a lesser extent beta-glucuronidase from *Escherichia coli* and egg white avidin, both of which are produced in maize. A number of experimental GM crops produce medicinal substances. Products that are “in the pipeline” include plants producing collagen, lactoferrin, lipase, aprotinin, vaccines, and monoclonal antibodies (Horn et al., 2004).

Although these crops have to be kept separate from the regular food supply, they may still be attractive for various reasons, including known production methods, exclusion of contaminations by certain biological agents or pathogens, storage without cooling of harvested seed, and the possibility to mix seeds to produce multi-component medicines (Streatfield et al., 2001). Another apparent advantage would be that the “impurities” of the medicinal product, *i.e.* the host food crops that have been modified, are “generally recognized as safe” in their own right. This would facilitate their safety evaluation for oral administration of the GM plant medicine. On the other hand, a disadvantage may be the fact that the glycosylation of glycoproteins in plants differs from that in other organisms.

Besides substances for medicinal purposes, GM food crops may also be modified with traits for other non-food purposes, such as industrial enzymes or traits that are focused on environmental clean-up, *e.g.* crops used for “bio-remediation”, *i.e.* the clean-up of environmentally contaminated soils.

In 2002, two incidents were reported in the US regarding commingling that might have had taken place between GM maize plants and conventional crops. The GM maize plants were found by the USDA Animal and Plant Health Inspection Service (USDA APHIS) in or near fields from which conventional crops had been harvested. This GM maize originated from seeds of experimental maize plants that had grown in these sites in previous seasons, producing a vaccine against porcine gastroenteritis. Thus, there was a potential risk that GM material had entered the regular food or feed chain (USDA APHIS, 2002; FDA, 2002a).

In popular press, it was reported that in both cases of potential contamination of conventional crops with experimental GM maize, the maize produced a vaccine against Swine Transmissible Gastroenteritis Virus (TGEV). The company had

previously reported its development of experimental GM maize expressing the S spike protein, a large surface glycoprotein of TGEV, and also reported positive results of a clinical trial on piglets with this product (Streatfield et al., 2001).

Following the incident, a number of actions were taken. For example, harvests potentially containing the GM maize were purchased in order to prevent their entry into food or feed. The US authorities, USDA and FDA, also issued new guidance on the safety evaluation and containment measures for GM plants that produce proteins for non-food purposes (FDA, 2002b; FDA, 2004). Among others, the authorities required that the safety of these crops for food uses be assessed at an early stage. Detection methods for the novel gene or protein were recommended as well as strategies to prevent contamination, by using marker genes that facilitate its distinction from other plants, or the expression in particular plant tissues. It should be determined at which points accidental commingling may occur. The harvested crop should not be processed in facilities where food or feed crops are processed without prior consultation. Waste streams should furthermore be inactivated. The guidance also described the data that needs to be provided on the molecular modification, the safety of the GM plant (*e.g.* allergenicity), and the quality of the medicinal products (FDA, 2002b). Some comments from non-governmental organizations and industry have been received in response to the publication of this draft guidance. Stricter measures were also imposed regarding the isolation of the experimental GM crops from conventional crops.

The biotechnology industry issued protocols regarding the prevention of commingling between experimental GM crops and conventional crops. Other stakeholders, including the Grocery Manufacturers' Association and the National Corn Growers Organization, also made suggestions as to how containment might be achieved, such as through geographical isolation or production in non-food or male sterile crops. More recently, in March 2005, the EPA introduced stricter rules for the cultivation of medicinal GM crops, such as the doubling of isolation distances towards neighboring crop fields and the use of strictly dedicated machinery (Becker and Vogt, 2005).

## 2.2. Botanicals

Botanicals include products such as herbs, spices and other plant-derived products, according to the work definition used for this review. This is in line with the definition of botanicals as provided by EFSA's Scientific Committee in its recent discussion paper on botanical food supplements (EFSA, 2004b). Botanical materials are thus defined as "whole, fragmented or cut plants, algae, fungi, lichens", and botanical preparations as having been "obtained from these materials by various processes (*e.g.* extraction, distillation, purification, concentration and fermentation)". This definition includes herbs, spices, and other plant-derived products.

Botanicals may be used for a range of applications, including uses as medicine or food supplements, both in humans and animals. It will therefore depend upon the application as to which regulation pertains to the botanical product. For example, EU regulations exist regarding the potential applications in food (including ingredients, additives, and colorants), human and veterinary medicine (including human traditional medicine), and feed (including feed ingredients and additives). Given the differences in requirements, it is suspected that botanicals sometimes may have other purposes additional to the ones that they are officially marketed for. The World

Health Organization (WHO) has also proactively pursued the harmonization of guidelines for testing the safety and efficacy of plant-derived pharmaceuticals including herbal medicines (WHO, 2000). At the European level, EMEA is responsible for the pre-market regulatory evaluation and supervision of human and veterinary medicinal products in the EU. Its Committee on Herbal Medicinal Products has released a number of documents on the safety of herbal products, including recent public statements on products containing allergenic soy or peanut proteins, *Capsicum*/capsaicin, *Chamomilla*, *Aristolochia*, estragole, methyleugenol, pulegone and menthofuran, or asarone. Apart from these regulatory issues, the market prospects for botanicals as human and animal health products are promising. For example, they may serve as an alternative to synthetic or biotechnological products whose use is restricted or prohibited, such as by the EU regulations on organic agriculture.

The following two cases pertain to recent incidents involving botanical products used for human applications. Because the background and chronological development of these cases are known, these cases can provide insights into how similar emerging risks of botanicals may arise in the future.

#### **2.2.1. Chinese herb nephropathy**

The first botanical case is about pills used as part of a slimming regime that has caused kidney damage due to the fact that the pills contained a nephrotoxic herb. This form of kidney damage is also referred to in literature as “Chinese herb nephropathy” (CHN). The presence of the nephrotoxic herb in the slimming pills has probably been due to the misidentification of the toxic herb as another commonly used herb. The kidney damage was first observed in 1992, in Belgian women who had been following the same slimming regime. A link was established between the complaints and the change in the formulation of a preparation containing herbs and other ingredients. One of the new ingredients was supposed to be *Stephania tetrandra*, but actually contained *Aristolochia fangchi*. The misidentification might have been due to similar appearances of these herbs growing in the wild and the similar names assigned to them in Chinese language.

The medical consequences of the intake of these herbs in the patients who reported themselves have been severe, with many patients undergoing dialysis or kidney transplantation. Depending on the dose, the toxic effects may have a protracted action, even after the intake of the noxious herbs has stopped. The number of known patients (105) is a fraction of the total number of persons who have taken these herbs (10,000), including the estimated number of persons who ingested sufficient amounts to be at risk of kidney damage (Nortier et al., 2000). In a number of known patients, kidney damage has progressed into kidney cancer. Analytical and toxicological studies have shown that the toxic effects, including carcinogenesis, relate to the presence of aristolochic acid, which can react chemically with deoxyribonucleic acid (DNA), creating genetic mutations (Arlt et al., 2000, 2001b; Cosyns et al., 1998; Nortier et al., 2000).

There has been some discussion as to whether other agents besides aristolochic acid might have been responsible for the induced toxicity. Most users of the slimming pills also used anorectic drugs such as fenfluramide and its active enantiomer dexfenfluramide (Martinez et al., 2002). However, in rats treated with

dexflenfuramid, the kidney histology did not differ from the controls (Debelle et al., 2003). Moreover, the morphological and biological features of CHN were strikingly similar to another fibrosing interstitial nephropathy, the Balkan endemic nephropathy (BEN). While aristolochic acid was incriminated as the cause of BEN, other authors considered it as an environmentally-induced disease strongly associated with the fungal mycotoxin ochratoxin A, which is also nephrotoxic, mutagenic, and carcinogenic. Aristolochic-acid-related DNA adducts in CHN patients were 50 times more abundant than those related to ochratoxin A (Arlt et al., 2001a), indicating that aristolochic acid was the most probable cause of CHN.

As the toxic effects became known, the herb preparations, whose formulation had contained *Stephania tetrandra* and *Magnolia officinalis* from May 1990 onwards, were withdrawn from the Belgian market in October 1992. The herb *Stephania tetrandra*, which had been mistakenly replaced by the toxic herb, was taken out of the formulation of the preparation. Investigations showed that the vacuum-packed, powdered preparations of *Stephania tetrandra* and *Magnolia officinalis* plants had been imported from China without any further quality control in Belgian pharmacies that dispensed the product (Vanherweghem et al., 1993).

Before and after the incident, similar incidents of intoxication with aristolochic acids from toxic herbs occurred outside Belgium, such as in Japan, the United Kingdom (UK) and France. In Japan and the UK, kidney affections were found to be linked to the consumption of products containing *Aristolochia manshuriensis*, a toxic herb related to *Aristolochia fangchi* (Tanaka et al., 2000; Lord et al., 1999).

Measures by the Belgian authorities included informing all general practitioners and chemists, and compiling of a list of dangerous herbs that were prohibited for use in herbal products in 1997 (Belgium, 1997). Other nations, such as Canada, Australia, Germany, and the UK followed this example by banning the use of herbs containing aristolochic acid (Kessler, 2000). In 2000, the Belgian authorities also issued a call for citizens and health professionals to report suspect cases of adverse effects from the intake of the slimming pills.

### **2.2.2. Intoxication by consumption of star anise tea**

The second botanical case is that of intoxications that occurred in a number of Dutch consumers of star anise tea in 2001. Within a short period of that year, a series of complaints were received by the Dutch National Food Inspectorate and the National Poison Information Center that were related to the use of star anise tea (Johanns et al., 2002). The tea was a brew made from a botanical mixture that contained, among others, Chinese star anise, or *Illicium verum*. This ingredient is made from typically star-shaped fruits that are imported from countries such as China and Vietnam. Contrary to the herbs used for the slimming pills discussed above, Chinese star anise does not grow in the wild, according to reports. Main areas of production of Chinese star anise are Southern China and Vietnam. Star anise produced in Vietnam is reportedly exported to China, where it is blended with Chinese produce for export to France (De Beer, 1993). Star anise for export is graded as "whole" or "broken". Fruits are packaged, among others, into bales or bags and exported from oriental seaports to third countries, where further processing can take place (e.g. GDV-TIS, 2005). The International Standards Organization (ISO) has formulated quality requirements for Chinese star anise, including the appearance of the fruits, as well as

their composition, number within 100 grams of star anise, and permissible level of imperfect fruits (ISO, 1995).

Besides the use of Chinese star anise for making tea brews, star anise is also used as a spice of flavoring in the Oriental kitchen. The US FDA considers Chinese star anise to be "generally recognized as safe" for use as spice or flavoring. Star anise may also be processed into oil with an anise-like taste, which is caused by the presence of anethole, which occurs also in other sweet tasting plants, such as European anise. The main producing areas for star anise oil are China and Vietnam (Iqbal, 1993). Some methods for identification of Chinese star anise and adulterations are actually based on the differences in oil composition between Chinese and other star anise varieties. According to the ISO standard for star anise oil, its *trans*-anethole content ranges between 86-93 percent (ISO, 1999). In this respect, it clearly differs from the oil component of Japanese star anise, which is rich in, for example, safrole and eugenol.

In the intoxications case, the Chinese star anise had been commingled with toxic, but similar looking fruits of Japanese star anise, *Illicium anisatum*, which are usually traded for non-food purposes, for example as decoration or as alternative medicine. Given that all these products may be called "star anise" may further add to the risk of accidental commingling (*e.g.* Small, 1996).

The toxic ingredients in Japanese star anise include anisatine, a neurotoxin, and myristicine, a hallucinogen. The symptoms that were related to the consumption of the suspect tea included epileptic reactions, such as convulsions, seizures, and aberrant brain activity (Johanns et al., 2002). Given that intoxications are a rare cause of epileptic seizures, physicians may not immediately link their patients' symptoms with this cause. Epileptic reactions caused by star anise occur with patterns of deviant brain activity, whereas other measures, such as serum chemistry, may be normal (Biessels et al., 2002; Ize Ludlow et al., 2004b; Johanns et al., 2002). Chinese star anise itself may also be toxic due to the presence of anisatine-like substances, such as veranisatine A, which also binds to  $\gamma$ -aminobutyric-acid receptors (Kuriyama et al., 2002). The levels and toxic potency of anisatine in Japanese star anise are higher than those of veranisatines A, B, and C in Chinese star anise (Nakamura et al., 1996). Therefore, Chinese star anise might also cause intoxications in infants, particularly at high intakes (Ize Ludlow et al., 2004b). Such high intakes may relate to the consumption of star anise in comparatively concentrated forms, in large volumes, or with high frequency. For example, Garzo Fernandez et al. (2002) performed a controlled clinical trial with the consumption of star anise infusion obtained from a low (< 5 fruits) or high (>5 fruits) number of fruits. A positive correlation was found between the level of intake and the occurrence of symptoms. Children especially appear to be sensitive towards the toxic actions of star anise. This is also relevant with regard to the popular use of star anise tea as treatment against colic in infants. Ize-Ludlow et al. (2004b) reported that doses of up to six stars of star anise boiled in variable quantities of water had been linked to the symptoms reported in infants.

Before the Dutch incident, intoxications with star anise had already been reported in other countries, including US, France, and Spain, among others through case reports in scientific medical literature (Garzo Fernandez et al., 2002; Gil Campos et al., 2002; Ize Ludlow et al., 2004b; Minodier et al., 2003).

After the first incidents in The Netherlands in 2001, a major product recall was undertaken and a public warning issued. The Food Inspectorate worked closely together with the National Poison Information Center with regard to reported intoxications, which necessitated hospitalization in some instances, *i.e.* for 22 out of 63 persons that had reportedly been intoxicated.

In 2002, the European Commission imposed measures requiring that consignments containing Chinese star anise were checked by exporting nations for possible commingling with Japanese star anise (Commission Decision 2002/75/EC). This decision was revoked one year later, given the absence of new incidents (Commission Decision 2003/602/EC). Outside the EU, authorities also took measures, such as the FDA in the US, which issued a warning in 2003, advising consumers not to consume brews derived from star anise. The FDA had been informed by the Florida Poison Control Center about cases reported by a Florida Children's Hospital (FDA, 2003; Ize Ludlow et al., 2004a). Also the Canadian government took preventive action by issuing a warning against star anise adulteration in 2004, based on accounts from abroad, without any intoxications being reported in Canada.

As regards prevention of intoxication with Japanese star anise, both types of star anise, which are similar in visual appearance, can be discerned from each other by microscopy and gas chromatography coupled to mass spectrometry (GC-MS; *e.g.* Saltron et al., 2001; Joshi et al., 2005). Also thin-layer chromatography (TLC) has been described by the European Pharmacopoeia as a method for quality control of Chinese star anise, which may be particularly useful for those laboratories that are not equipped with advanced analytical apparatus for certain microscopic methods or GC-MS.

### **2.2.3. General risks related to herbal medicines**

The rising popularity of these medicines may also bring with it some problems of adverse health effects related to, for example, misidentification of herbs, lax quality control, and contamination.

As regards the production of herbal medicine, two methods can be distinguished, *i.e.* collection of specimens either of plants growing in the wild or of cultivated plants. The trade in herbs usually involves the intervention of brokers, which can cause loss of data on the specific origins of the product. This pertains especially to businesses that obtain their inputs from the open market. Other businesses may have internalized the whole or parts of the production and processing of herbs (*e.g.* Green, 2002). These characteristics may have their bearings upon the quality and information on the products being produced or traded. Ideally, information management within the production chain is integrated with quality control systems, such as in Effective Resource Planning (Groot and Van der Roest, 2006). The implementation of quality control systems may also pose specific requirements, such as in developing countries (Shankar and Venkatasubramanian, 2005).

Eastern Medicine, such as traditional Chinese or Ayurvedic medicine, may bring with it characteristic risks related to quality problems associated with enhanced production for exports, complexity of the traditional medicine systems, and differences in standards. As regards quality control of traditional medicine, it should be taken into account that traditional knowledge may provide valuable inputs regarding the most

optimal conditions for growth and intrinsic characteristics of the medicinal plant (Shankar and Venkatasubramanian, 2005).

General risks associated with herbal medicines include:

- Misidentification, which can be prevented by methods such as designation with Latin names. Potential causes of misidentification include contamination of cultivated herbs with weeds, and resembling plants mistaken for herbs collected in the wild;
- Contamination with noxious substances, such as heavy metals, polycyclic aromatic hydrocarbons, dioxins, and PCBs, as well as natural toxins, or micro-organisms (*e.g.* Ko, 2004)
- Interaction with other medications, such as antagonism or synergism, and with medical tests, potentially leading to misdiagnosis (*e.g.* Hu et al., 2005; Scott and Elmer, 2002; Strader et al., 2002)
- Adulteration with other pharmaceuticals (Corns, 2003)
- Intrinsic toxicity due to the presence of natural toxins, such as the aristolochic acids mentioned above (Chan et al., 2005).

Some botanicals used as herbal medicines, particularly dry herbs, may also find their way into other applications, such as spice or tea. If herbs are used for medicinal purposes, they should fulfill the same safety requirements as regular medicines.

### 2.3. Residues of antibiotics in farmed shrimp

In the last few decades of the previous century, the cultivation of shrimps in confinement, commonly referred to as shrimp aquaculture or shrimp farming, has steadily increased in South East Asia, making it an important economic activity in some regions (FAO, 2005). Furthermore, in order to meet increasing demands, intensification of shrimp aquaculture has been stimulated by local institutions. The shrimp sector is also important for exports from these countries to other markets, in which globally operating companies play an important role. As a corollary to the rapid rise in production, antibiotic use has also increased in order to prevent losses due to diseases (*e.g.* Lebel et al., 2002).

The use of antibiotics in shrimp aquaculture may have accounted for the detection of residues of the antibiotic CAP in shrimps imported from Vietnam and China into the EU in 2001. In the period after this, more findings of CAP and nitrofurantoin antibiotic residues in shrimps and in some cases also in other fishery products and poultry followed. These findings led the EU to impose temporary controls for the presence of antibiotics on all imported shrimps from China, Vietnam, Thailand, and Indonesia during 2001 and 2002 (Decisions 2001/699/EC, 2001/705/EC, 2002/250/EC, and 2002/251/EC), and the subsequent suspension of imports from China (Decision 2002/69/EC). Based on the results of detection and the preventive actions imposed and guarantees provided by the exporting nations, the EU subsequently revoked these measures in the period 2002-2004 (Decisions 2002/770/EC, 2002/994/EC, 2003/477/EC, and 2003/546/EC). The underlying reason for the EU measures was the “zero tolerance” policy towards the CAP and nitrofurantoin antibiotics because no internationally harmonized threshold levels, such as an acceptable daily intake (ADI) or maximum residue level (MRL), existed for these substances. This was due to incomplete information on the toxicity of these compounds, so that international experts advising Codex alimentarius have been unable to establish such thresholds.

Other authorities besides the EU, such as the US FDA, also had increased their checks of imported shrimp for antibiotics.

The issue of antibiotics used in shrimp farming and their potential presence in shrimp products destined for the international food market has been chosen as an example of an emerging risk for various reasons. Data are available on the scientific background of the problem, as well as on the risk management measures that have been taken. While awareness of the use of the CAP and nitrofurantoin antibiotics has been heightened by the incidents, this case study may aid the early identification of similar, future problems in aquaculture products. This is because besides shrimp, other species produced through aquaculture may face similar issues. Antibiotics may also still be readily available and provide a cost-effective means towards disease management in many countries. Besides that, the detection of antibiotics may also become increasingly likely through the application of increasingly sophisticated and sensitive analytical methods such as liquid chromatography with MS detection. Such discoveries trigger inspections of samples for the presence of these antibiotics on a wider scale, further enhancing the likelihood of findings of residues. Furthermore, the incidents have had far-ranging consequences for the shrimp industries in the exporting nations and have received media attention in the importing regions, potentially affecting public perception of food safety in general. The relevance of this topic is also recognized by policy makers, as illustrated for example, by the bilateral projects between Holland and several Asian shrimp-exporting countries to seek possible alternatives to antibiotics. Moreover, the European Commission has intensified disease monitoring and prevention in fish cultivation systems, and harmonized legislation on aquaculture (European Commission, 2005).

### **2.3.1. Shrimp farming and treatment of diseases**

The major causes of disease in farmed shrimp are the bacterium *Vibrio* spp. and viral agents. The pathogens may be attracted from the surface water used for farming in which they naturally occur. Generally, the resistance of *Vibrio* and some other pathogens to CAP is absent or low, providing CAP as an attractive option for treatment of diseases caused by these pathogens (*e.g.* Otta et al., 2001). Besides therapeutic treatment, antibiotics may be used for preventive purposes and are commonly mixed with the shrimp feed before their application (prophylactic application). Due to differences in the prohibition of antibiotics between nations, the use of some antibiotics in seafood farming may have been prohibited in importing nations whereas their use has previously been possible in exporting nations. There have also been accounts of the illegal use of antibiotics. As an alternative to antibiotics, prebiotic and probiotic preparations are reportedly being used. Although antibiotics cannot eradicate viral diseases, they still may be applied for this purpose due to lack of awareness among users. Frequent use of antibiotics has also caused an increase in antibiotic resistance in bacteria and may therefore also constitute a food safety issue if human pathogens are involved (Alderman and Hastings, 1998; Holmstrom et al., 2003).

The hypothesis has been formulated that low levels of antibiotics may derive from environmental sources due to dissemination after medicinal use and to natural occurrence of these antibiotics, such as CAP that is naturally produced by *Actinomyces venezuelae* (Hanekamp and Wijnands, 2004). But the Joint FAO/WHO

Expert Committee on Food Additives (JECFA), however, concluded that this was an unlikely scenario for CAP (JECFA, 2004).

In exporting nations, dedicated export agencies commonly examine shrimps destined for exports before their shipment to overseas ports. At this stage, shrimps may already have undergone forms of processing, including washing, drying, decapitation, peeling, deveining, and boiling. Usually, imported tropical shrimp have been peeled, frozen, and shipped, and have to undergo further processing, such as boiling. Further processing, if applicable, may also yield shrimp meal as substrate for shrimp crackers, as well as chitin as a by-product of peelings. The application of “hazard analysis critical control points” (HACCP) principles to the shrimp processing industry minimizes the risk of contamination in this stage of production.

### **2.3.2. Consumer safety**

The most likely route of exposure of consumers to antibiotic residues present in products of aquaculture is through oral uptake. Prior to consumption, the product may have undergone processing, and after consumption, the digestive process may have an impact on the availability and effects of the antibiotic. Consumers may also have been exposed to the same antibiotics through other routes, such as through medicinal use and, in the case of CAP, via an eye treatment solution.

Both CAP and nitrofurans are rapidly absorbed from the gastrointestinal tract after oral uptake and distributed to all organs (*e.g.* INCHEM, 1997; JECFA, 1993a,b; 1994; 2004). The intestinal microflora also metabolizes CAP to various metabolites. CAP is mainly excreted as glucuronide through the urine. Therapeutic doses of CAP may cause depletion of blood cells (aplastic anemia), depression of bone marrow cell production, and ultimately leukemia. Children appear to be particularly sensitive to these effects. Although depletion of blood cells is a rare event, the consequences may be lethal. There appears to be no link between the use of CAP for eye treatment and depletion of blood cells. JECFA (1994) concludes that the same level of intake through food as for eye treatment is therefore also unlikely to cause depletion of blood cells.

Codex alimentarius has considered a number of nitrofurans, including furazolidone, nitrofurantoin, and nitrofurazone (nitrofur). While acute lung toxicity, carcinogenicity, genotoxicity, and mutagenicity have been observed in animals in a number of cases, Codex alimentarius was unable to establish an ADI or MRL because the available data were not sufficient. Supplementary data were also needed on the chemically bound residues of metabolites of furazolidone in tissues (JECFA, 1993ab; INCHEM, 1997). It can also be difficult to extrapolate these effects to low-dose intakes that might occur during food consumption. A risk assessment of CAP in imported shrimps estimated, for example, that the intake of CAP by consumers from food would be by hundreds of millions times less than to therapeutic doses (Hanekamp and Kwakman, 2004). It is currently also considered that metabolites of nitrofurans may be toxic as well in some cases (BgVV, 2002).

Because no ADI/MRL exists for CAP and nitrofurans, as explained above, some nations, including the EU, exerted a "zero tolerance" policy towards these compounds. Any presence of residues of these veterinary drugs in foods above the limit of detection would therefore have been considered a contamination. This should

be viewed against the background of the development of increasingly sensitive analytical methods which can detect minute quantities of these antibiotics (FAO/WHO, 2004).

In the course of the events surrounding the presence of unauthorized antibiotics in imported shrimps, the EU imposed "minimally required performance levels (MRPLs)" for compounds without ADI/MRL (Decision 2002/657/EC). MRPLs constitute the lowest levels that can reliably be considered "non-zero" in the enforcement of "zero tolerance." A later decision of the EU defines these MRPLs as action levels for residues of specified chemical compounds (Decision 2005/34/EC). If consignments of products contain levels of these compounds that exceed the MRPL, they should either be destroyed or shipped to a non-EU country, provided the latter grants its approval for import. Examples of MRPLs in aquaculture products include 0.3 ppb for chloramphenicol and 1 ppb for nitrofurans (furazolidon, furaltadon, nitrofurantoin, nitrofurazon). Prior to these EU-harmonized measures, some Western countries had imposed different levels-of-detection for the same antibiotic in the initial phases of the incidents.

A workshop organized by the Food and Agriculture Organization (FAO) and WHO in 2004 retrospectively considered the issues surrounding developments in 2001-2002 that led to the findings of antibiotic residues in imported products and the measures that followed. The recommendations that were thus formulated included the prioritization of certain substances for definition of Codex MRLs, as well as the use of national MRLs as temporary Codex MRLs. A "threshold of toxicological concern" may be applied as well (FAO/WHO, 2004).

### **2.3.3. Factors contributing to the incident**

A number of factors contributed to the development of the incident. Some of these factors, such as antibiotic use, probably existed before they were recognized as problem. The following factors have thus been identified:

- Lack of internationally harmonized legislation and quality assurance. Due to incompleteness of fundamental toxicity data, no harmonized threshold data could be formulated by Codex alimentarius. Without ADI/MRL, importing nations pursued a "zero tolerance" policy towards the topical substances. Also the organization of the production and trade chains, including a lack of quality assurance (QA), communications, and synergy between partners in some parts of the system may have compounded the control of the usage and presence of antibiotics. Awareness of regulations and appropriate usage of antibiotics may also have been low among some producers.
- Zero tolerance and increasing sensitivity of analytical methods. With zero tolerance, any reliable positive detection of antibiotic residues leads to refusal of the pertinent consignment. With increasing sensitivity, the ability of detecting very small quantities of antibiotics increases, and so does the likelihood of finding residues. If antibiotic residues can originate from sources other than those used in aquaculture, such as from their presence in the environmental background, this has to be taken into account.
- Partially linked with the previous point is the risk of detection of false positives. For example, the analysis may target a compound originating from multiple sources, necessitating careful interpretation of the analytical results in order to avoid false positives. Known examples include semicarbazide, which

is a metabolite of nitrofurazone, but also a product formed from azodicarbonamide used as blowing agent in production of plastic food contact materials or as food additive in cereal flour. Another example is aminomethylphosphonic acid (AMPA), which is an environmental microbiological breakdown product of the herbicide glyphosate, but may also derive from certain detergents.

- Illegal use of – or easy access to – antibiotics. The presence of residues due to illegal use of CAP and nitrofurans currently appears a remote possibility because of the measures that exporting nations have imposed, as well as because of the import controls imposed by receiving nations. It should be cautioned that this pertains to the nations and products at the focus of the incident discussed in this publication. Meanwhile, conditions still exist that may facilitate the use of new, alternative antibiotics, including the non-regulated status of antibiotics in some nations and the ready availability of antibiotics in nations that produce these compounds, for example.
- Distinctly increasing production and trade. An important factor contributing to increased disease pressure in aquaculture is increasing trade in (hence production of) shrimp, as previously observed in Asia. This is because increased production is commonly achieved through intensification of aquaculture. This intensification is usually followed by increased disease pressure in the system within several years. This cascade of events probably also has triggered the emergence of the problem in the Asian culture of shrimp. These conditions may be further aggravated by lagging financial support from local authorities, despite the incentives to farmers to set up aquaculture in a relatively short period, making antibiotics a cost-effective option to treat the problem. Shrimp farmers may also be prone to search for new compounds which are not regulated either, as alternatives to prohibited antibiotics.

#### **2.4. Case study on pesticides of natural origin**

Pesticides of natural origin provide an option for disease prevention and management in agriculture besides synthetic pesticides and disease-resistant crop varieties, for example in cases where resistance to synthetic substances has arisen.

Sudakin (2003) divides bio-pesticides into 3 classes: 1) microbial pesticides like bacteria, fungi, algae, viruses, and protozoa; 2) genetic incorporation of DNA in plants to protect against insects; and 3) biochemical pesticides that consist of naturally occurring chemicals that are active against pests. This review considers the first and third classes, whereas the second class comprising GMO-based pesticides falls beyond the scope of this review.

The first class of pesticides may consist of living micro-organisms, which may either antagonize or parasitize the target pest, such as through the production of toxins with a narrow activity spectrum (Montesinos, 2003). These pesticides can also be used for selective protection of plant infection wounds, interfering with the pest's attacking approach through competition for nutrients and space (Raaijmakers et al., 1995). Distinctive features of these pesticides are their narrow target range, specific and slow mode of action, relatively critical application times, suppressive rather than eliminating character, limited persistence and a short shelf life. Moreover, they apparently present no problems of chemical residues (Dewhurst, 2001). Viable

micro-organisms still have the capacity to grow, reproduce themselves, and/or adapt their physiology according to the environment that will surround them after their application. This contrasts with the behavior of non-viable residues, whose activity decreases with time. The behavior of biological pesticides in the first class therefore may be less well characterized than non-viable residues, thereby posing an emerging risk. An example of this class is pesticides based on *Bacillus thuringiensis* (e.g. Bellinger, 2005).

The third class of pesticides is commonly produced from plants and minerals. For example, a commercially available plant-extract-based pesticide is neem oil, which is obtained from a tropical tree and which is discussed further below (Higgins, 2001). This class of pesticides may generally be perceived as being less toxic than synthetic ones, but it should be borne in mind that their application may lead to the introduction of unnaturally high levels of natural toxins, which may cause adverse effects. Furthermore, these substances mostly have a shorter half-life than synthetic ones (Duke, 1990).

From a general perspective, pesticides are regulated by national and regional legislation, such as EU Directive 91/414/EEC, under which permits are granted to the use of these substances, usually as part of formulations for use on specific crops. The EU is currently re-evaluating the pesticides that have previously been allowed onto the market in the EU, and estimates predict a decrease of 900 existing products in 1991 to less than 400 in 2008. For petitions to register a pesticide, an extensive dossier has to be submitted, which should contain scientific data on toxicological studies in animals, ecotoxicology, traceability, and ecological impact (Montesinos, 2003). Pesticides of natural origin have to comply with these requirements as well. Besides regulations for general pesticides, regulatory provisions may pertain specifically to natural pesticides. For example, the EU has made amendments to its pesticide legislation in order to include biological pesticides such as micro-organisms (Directive 2001/36/EC). Application dossiers submitted under this legislation should provide data on the micro-organisms' species, basic biology, and selection procedures, as well as data on assessments of risks for human health, efficacy, and environmental impact. Furthermore, regulations on organic farming in- and outside the EU (e.g. EU Regulation 2092/91/EEC) prohibit the use of the most available pesticides for this kind of farming, which therefore has to rely on a comparatively small group of pesticides for sustainable yield and quality of the crops. Pesticides of natural origin, whose use aligns with the principles of organic farming, may provide important alternatives to this sector, and the regulatory environment is therefore important factor for their safety and availability.

The generally held presumption that natural means safe does not always hold true. Some natural pesticides are indeed non-toxic to humans, while others are as toxic as- or more toxic than- many synthetic chemical pesticides. The toxicity of these pesticides may be unexpected because of intrinsic factors or due to different behavior in the environment, and these pesticides therefore may constitute an emerging risk in their own right.

The natural pesticides neem and rotenone are discussed below as examples of possible risks associated with this class of pesticides. As regards neem, this product has a record of apparently non-toxic behavior and long traditional use, but modern

production methods may introduce new toxic ingredients that may give rise to emerging risks. With regard to rotenone, there have been accounts of an assumed relationship between rotenone and Parkinson's disease.

#### **2.4.1. Neem-derived pesticides**

The neem tree [*Azadirachta indica* A. Juss. (Meliaceae)] naturally occurs in Africa and India and is a traditional source of crop protection agents, including, for example, extracts or leaves that are traditionally used for the protection of harvested kernels or beans against insects. The biological effects of neem products include repellency, feeding deterrence, growth disruption, reduced viability, and disturbed reproduction. Bitter compounds that act as insect anti-feeding or hormonal disruptors are found in all parts of the tree. Of these compounds, azadirachtin is the most active insecticide, which is predominantly found in the seed (Schmutterer, 1990). Neem extracts are also used against moulds in crops because of the antifungal activity of these extracts (Subapriya and Nagini, 2005).

Unprocessed neem or derived neem seed oil are used for the protection of harvested and stored seeds intended for food purposes, which therefore they may contain neem residues. Because seeds have to be kept dry, the use of aqueous extracts is not recommended and instead, non-aqueous extracts are used that probably leave little residue on these seeds (Boeke et al., 2004).

The risk of ingestion of unprocessed neem material from residues on beans by humans has been estimated by Boeke et al. (2004). For the crude neem powder product, the powder residues on unwashed beans would exceed the calculated safe dose, while sieving or washing off removes most of the powder. No reports exist on the level of exposure during incidents involving toxicity of the crude material in humans. Moreover, ground or dried leaves and fruit kernels are applied immediately without further processing that may concentrate toxic components, such as by extraction or isolation of seed oil. Neem-oil-treated beans may contain residues, of which the levels are likely to be much lower than those corresponding to intakes causing acute toxicity in laboratory rodents. Based on data on effects on fertility in a sub-acute exposure study in female rats, these authors propose an ADI of 0.002 ml neem oil/kg bw, which is far below the levels that have shown toxicity in children (0.2 ml/ kg bw). Furthermore, the pure neem compound azadirachtin is relatively non-toxic and has an oral subchronic NOAEL of 1500 mg/kg bw in rats (studies cited by Boeke et al., 2004). There is apparently no risk of residue formation through environmental dissemination because neem residues are readily degraded in the environment, although some aquatic organisms are sensitive towards the toxicity of neem products.

A number of studies have been carried out on the toxicity of neem products in laboratory rodents, besides other studies that have been carried out in domestic animals and wildlife organisms in ecotoxicology studies, as well as studies on physiological effects. For example, neem leaf extracts are toxic in mice and guinea pigs, leading to gastro-intestinal spasm, hypothermia, inhibition of thyroid function, genotoxicity, and death (Awashy, 2001; Boeke et al., 2004). Neem bark aqueous extract appeared to be safe in rats, but lethal in snails and some fish (Osuala and Okwuosa, 1993). An aqueous extract of neem seed kernel inhibits trypsin activity in weanling rats (Jacobson, 1995).

In experimental animals, neem oil exhibits acute toxicity in rats and rabbits with LD50 values of 14 and 24 ml/kg, respectively. Target organs are the central nervous system and lungs (Brahmachari, 2004). Also commercial neem products such as Nimbidin, Vepacide, Margosan-O have been toxicologically tested. Daily administration of Nimbidin, for example, caused subacute toxicity in rats at 25-100 mg/kg bw, while the compounds sodium nimbidate (intraperitoneal LD50 in mice of 700 mg/kg bw), nimbolide (intraperitoneal LD50 in mice of 225-280 mg/kg bw) and nimbic acid (intraperitoneal LD50 in mice of >600 mg/kg bw) can be lethal or cause dysfunction or damage of various organs (Kanungo, 1996, and Murthy and Sirsi, 1958, both cited by Brahmachari, 2004; Glinsukon et al., 1986). Vepacide, another neem based pesticide, caused changes in enzyme profiles indicating liver necrosis at all doses ranging between 80-320 mg/ kg bw (Rahman et al., 2001). By contrast, azadirachtin, which is the active ingredient of Margosan-O, is short-lived, easily degradable, and well-tolerated by rats (oral NOAEL 1500 mg/kg bw) and mice (Jacobson, 1995; Raizada et al., 2001).

Besides experimental animals, in some cases, neem oil has also been reported to produce toxic effects in humans, leading to toxic encephalopathy, especially in infants and young children. Symptoms are vomiting, drowsiness, tachypnoea, and recurrent general seizures (Lai et al., 1990). However, local application of 1% neem oil in children and adults has not revealed adverse effects after 1 year of exposure (Valecha et al., 1996). For humans, the estimated daily safe dose of non-aqueous extracts, like for acetone extract (12.5 µg/kg bw) is low compared to the safe doses of unprocessed neem (0.26 mg/kg bw), aqueous extracts (0.3 mg/kg bw), neem oil (2 µl/kg bw), and pure azadirachtin (15 mg/kg bw; Boeke et al., 2004). Probably other compounds besides azadirachtin are responsible for this toxicity, but these toxic components need to be further scrutinized before more definite conclusions can be reached on the safety of neem extracts (Boeke et al., 2004).

#### **2.4.2. Rotenone and possible contribution of pesticides to Parkinson's disease**

The reason for choosing the commercial, natural pesticide rotenone as a case is that in 2000, it received media attention as a possible factor contributing to the development of Parkinson's disease (e.g. CNN, 2000). Furthermore, the UK-based organic farmers' organization Soil Association posed restrictions on the use of rotenone by applicators, although workers were considered to run only a low risk and consumers none at all (Soil Association, 2005). These events occurred after a scientific study had been published in which rats received rotenone by infusion into their blood stream and subsequently developed symptoms and features typical of Parkinson's disease (Betarbet et al., 2000). Before this discovery of the possible effect of rotenone on Parkinson's disease, toxicity data for this chemical pertained to, for example, general toxicity and carcinogenicity (EXTOXNET, 1996).

Rotenone is derived from roots of plants, such as *Derris elliptica* and *Lonchocarpus utilis*, which produce high levels of the compound (Soloway, 1976). The insecticidal properties of rotenone are exploited, for example, in home gardening, organic farming, and topical application on animals. It is used in many countries, such as in the EU, where it has been approved under Regulation 2092/91/EEC on organic farming. Besides its use in agriculture, there are accounts of its use in some nations as a chemical to eliminate fish.

Parkinson's disease is a neurological disorder whose patients have impaired motor activity, such as movement of limbs. On the histological level, this impairment is accompanied by degeneration of brain parts known as *substantia nigra* (black substance) and *striatum*, while on the microscopic level cells in these parts contain inclusions of incorrectly folded neuronal proteins. Mutations in the genes for these proteins are among the genetic factors known to predispose for the development of Parkinson's disease. However, in elderly people, the development of this disease appears to be linked to non-genetic factors (reviewed by Langston, 2005).

The mechanism of action of rotenone involves the inhibition of a mitochondrial enzyme, NADH dehydrogenase, leading to oxidative stress, which can be harmful to cells.

In rats used in the rat experiment mentioned above, intravenous administration of rotenone at a dose of 2-3 mg/kg per day during week 1 - 5 led to partial inhibition of mitochondria in the brains of the rats, and also to changes in the brains of nearly half of the animals which typically also occur during Parkinson's disease, such as formation of lesions, occurrence of cytoplasmic inclusions, and loss of the neurotransmitter dopamine. The behavior of animals whose brains had been affected was also typical of Parkinson's disease (Betarbet et al., 2000).

The rationale for the rat experiment was that rotenone was a known inhibitor of mitochondrial complex I, similar to N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which was a compound known to cause Parkinson's disease (Friedrich, 1999). MPTP had previously been identified as a cause of drastic development of Parkinson's disease in drug addicts who had taken synthetic heroin contaminated with MPTP (reviewed by Langston, 2005). MPTP is metabolized to the reactive compound 1-methyl-4-phenylpyridinium (MPP<sup>+</sup>), which is taken up selectively by dopamine-producing brain cells where it can cause inhibition of complex I and damage leading to Parkinson's disease. Contrary to MPP<sup>+</sup>, rotenone does not need to be taken up actively by brain cells because as a lipophilic compound, it can pass cell membranes.

In addition, a number of epidemiological studies indicate that there is a link between previous exposure to pesticides and the development of Parkinson's disease in later life. These studies, however, cannot trace the source back to a specific pesticide (reviewed by Kamel and Hoppin, 2004). Interestingly, the pesticide paraquat shows structural resemblance to MPP<sup>+</sup> and is known to cause oxidative stress and damage to dopamine-producing brain cells (Przedborski and Ischiropoulos, 2005). Pesticides like rotenone, paraquat, and dieldrin also stimulate formation of protein fibrils that can form part of typical inclusions in these cells. Besides pesticides, exposure to other environmental factors, such as microorganisms and previous infections, may also be linked to the development of Parkinson's disease (Langston, 2005).

Despite the outcomes of the above mentioned rat and laboratory studies, no neurotoxic symptoms in rotenone-exposed mammals via topical application have been reported in the literature. The results of relatively high doses of rotenone administered intravenously to rats should therefore be interpreted and extrapolated with caution. Human exposure to rotenone will primarily be through inhalation and dermal contact by pesticide applicators and ingestion by consumers of contaminated

foods. For these routes of exposure, intravenous administration is not a realistic model. Moreover, rotenone is relatively non-persistent; sensitive to heat, light and oxidation; poorly absorbed during digestion in mammals and does not accumulate in animals. Prolonged oral administration of rotenone to rodents does not cause adverse effects on the brain (*e.g.* at oral doses up to 75 mg/kg bw in rats during two years), while accounts of severe human intoxications are scarce (*e.g.* reviewed by Ling, 2003).

### **3. Lessons learned**

#### **3.1. Unauthorized GM products**

Several cases have been described above in which the commingling of GM crops that had not been authorized for food use with conventionally grown crops destined for food use occurred. This has triggered measures to prevent potentially commingled consignment from entering the food supply and also to prevent similar incidents in future. It can be argued that if the commingling had been detected in an earlier stage, this would have prevented costly measures. In the case of other non-authorized GM products, however, there may be no appropriate detection methods available for these GM products.

The case of GM crops with medicinal traits described above highlights some interesting features regarding regulatory and private measures. For example, the US authorities undertook action based upon indications that certain commingling might have occurred rather than upon the actual detection of commingling. In this sense, the measures were precautionary.

The recommendations regarding the early detection and prevention of emerging hazards similar to the examples described here include:

- To introduce multiple risk management measures in the country where the GM crop is grown, including regulations that amongst others cover the food safety of GM crops, also if these crops have non-food purposes, as well as enforcement, containment, and traceability;
- To create a database with data on the experimental and commercial GM crop varieties that are grown worldwide in the field. These data should include, for example, data on the introduced traits, detection methods, reference materials, and safety assessments. Additional "foresight" studies of new developments in gene technology may be undertaken with particular focus on those GM crops that may become a health problem; and
- To carry out research on the mechanisms of gene flow between plants, and to monitor unauthorized GM crops in and around experimental fields.

#### **3.2. Botanicals**

In the case of CHN and star anise tea intoxications, the botanical with an experience of safe use by consumers had been mistakenly replaced with another toxic botanical with similar appearance. A number of factors have been identified that might have contributed to the misidentification of the toxic variant for the commonly consumed one, such as similar sounding names and similar shape of plants collected in the wild. Another contributing factor may have been the market availability of both the safe and toxic variants, the latter for non-food purposes, such as in the case of star anise.

Preventive measures that can be taken to prevent this accidental misidentification of botanicals from recurring include:

- Appropriate labeling of botanical products, among others by displaying the Latin botanical names of the ingredients;
- Screening for the presence of toxic constituents of herbs in botanical preparations, such as heavy metals, ephedrine-type alkaloids, and aristolochic acid. Screening methods, such as TLC, and confirmatory methods, such as high-performance liquid chromatography coupled to MS detection, have been described in scientific literature (*e.g.* Sullivan and Crowley, 2006).

It also appears from the success of the EU measures that the introduction of chain and quality management by exporting nations can be also effective in preventing contamination.

Based upon the experiences learned from the two cases described above and the risks of herbal medicines in general, the following recommendations are made:

- Be more prepared for incidents by raising awareness and by (further) introducing quality systems and standards. For example, compile a list of botanicals that regardless of their application should comply with minimal quality criteria, and other botanicals that should be prohibited given the presence of intrinsic toxic substances (for example, aristolochic acid).
- Carry out pro-active identification of botanicals that may become a problem in future, based upon factors such as potential for admixture with toxic species and the presence of known toxic components.
- Take into account, in a broader sense, the potential presence of toxic plants in other products, such as weed seeds in agricultural harvests. Different agricultural practices, such as herbicide use, may lead to different levels of contamination.

### 3.3. Residues in aquaculture products

The developments prior, during, and after finding of CAP and nitrofurant antibiotic residues in imported shrimps as described above provide a view on how the emergence of this issue as a new risk has occurred. This emergence cannot be pinpointed to a single cause, as it appears to have developed from an interaction between many factors. These factors can serve as indicators for the prediction of the probable emergence of similar problems in future.

In order to prevent recurrence of similar problems with other chemicals used for disease management in the future, it is recommended that pro-active risk management be carried out. The following measures may support such a pro-active approach:

- Education and quality control. To endow the local industry with the appropriate back-up and skills, large-scale education programs for individual shrimp farmers may be needed (*e.g.* NACA, 2002). By stimulating the introduction of HACCP-based systems, the unwanted introduction of prohibited antibiotics and other hazardous chemicals can be controlled better. In more general terms, farm management practices can help prevent the introduction of diseases from any other external sources and their transmission from farm to farm. An example of awareness-raising activities in the South East Asian region is the “road show” that has been set up by the Asian and Dutch authorities (Dutch MoA, 2005).

- Implementation of stringent regulations. In some instances, antibiotics may be easily obtained and their use not registered in countries where shrimps are cultivated. Regulatory requirements for mandatory registration of medicines including antibiotics will help controlling the distribution chain. The exporting and importing nations' analytical capabilities should also be attuned to each other with regard to MRPLs in case of "zero tolerance", which may be stringent.
- Pro-active identification of potential future hazards. Based on the characteristics of the antibiotics that have already been identified as problematic in shrimp aquaculture, other substances with similar characteristics may be identified. Geographical areas where problems are likely to emerge in the near future may also be identified.

These recommendations not only apply to the shrimp industry, but could be extended to all aquaculture industries, since the problems and challenges are also found in these industries, especially in developing countries.

Whilst the case study above focuses on antibiotic residues, also other food safety issues may be particularly linked to aquaculture practices. Another recent incident pertaining to contaminant residues in aquaculture products, for example, was that of organochlorine (dioxins, pesticides) residues in farmed salmon. This incident has been reviewed elsewhere in more detail (Kleter et al., 2006a). The incident followed a publication of a study amongst cultured and wild salmon (Hites et al., 2004a). It was thus observed that the residues of organochlorine compounds were generally higher in farmed salmon than in its wild counterpart. Whereas regulatory measures and scientific knowledge underpinning these measures had already been well-developed, consumers responded negatively in some countries (reviewed by Kleter et al., 2006a). This incident therefore showed the potentially important role of diverging interpretations of scientific results and risk communication in the development of incidents over food safety. Interestingly, a more recent publication of the same group of authors focused on contamination of farmed salmon with similar contaminants, *i.e.* polybrominated biphenyls derived from flame retardants (Hites et al., 2004b). This showed that the results of the previous study on organochlorine residues could be translated to contaminants of a similar nature.

More generally, a recent report maps the salmon production chain and reviews trends in hazards within this chain (Van der Roest et al., 2007). Interestingly, this report also highlights the rapid decrease of antibiotic use in Norwegian salmon aquaculture following the introduction of salmon vaccination and stringent regulations prohibiting the use of antibiotics. These authors also identify a trend towards plant-derived alternatives for the use of fish-derived ingredients in fish feed, which on one hand may diminish hazards such as the contamination with organochlorine compounds mentioned above, but on the other hand may also introduce new hazards (Van der Roest et al., 2007).

### **3.4. Pesticides of natural origin**

There is an increasing interest in the use of pesticides of natural origin for various reasons. To date, most pesticides used to protect crops are synthetic compounds, whereas only few are biological agents. Two examples of natural products used as pesticides have been discussed in more detail above, *i.e.* products derived from neem tree and rotenone.

While neem is an effective crop protection agent for post-harvest use and potential health effects of residues have not been reported yet, more investigation is needed. The striking difference in toxicity between aqueous and non-aqueous extracts warrants further investigation, especially because the component in the non-aqueous extracts that causes toxicity in laboratory rodents is not known. New extraction methods used for traditional safe botanical pesticides may thus give rise to the introduction or concentration of this unknown component and hence create new and unexpected risks.

As summarized above, research has shown a correlation between intravenous administration and neurotoxicity of rotenone in rats, although this rat experiment has not been representative of the likely low exposure of humans to rotenone *in vivo*. In more general terms, this and other recent research findings have increased the awareness among the scientific community of the potential neurotoxicity of some environmental contaminants, both natural and synthetic, with similar molecular mechanisms of action (*e.g.* reviewed by Drechsel and Patel, 2008).

The following generalized recommendations are based upon the general considerations and the cases of neem tree and rotenone in particular:

- Attention should be given to the fact that traditional, safe, plant-extract-based pesticides may form an emerging risk when new extraction methods are applied.
- An equal approach towards the regulatory risk assessment of natural and synthetic pesticides with known or anticipated neurotoxic properties should be followed.
- Biological pesticides containing living organisms may present a new type of hazard both for the environment and public health due to their ability to grow, reproduce themselves, and adapt their physiology according to the receiving environment, and therefore need further investigation.

#### **4. Overall conclusions**

The case studies on biochemical and chemical risks for food safety described above all had their particular features. Nevertheless, the conclusions for these specific sections included recommendations that might be relevant for future incidents with similar chemical risks. Besides these case-specific recommendations, the following generalized conclusions from these case studies can be drawn:

- There is a need for databases on which contaminants (toxic herbs, GMOs) might be present within certain types of products. This may be especially valuable in cases of international trade where the receiving country and exporting country have different productive and regulatory environments (for example, for GMOs that are allowed onto the market in the exporting country, but not in the importing country).
- Pro-active "reconnaissance" can help identify products at risk of being contaminated. For this purpose, criteria or indicators should first be formulated that can facilitate the identification. The previously mentioned databases can aid the pro-active search for potential products at risk (or *vice versa*, the search outcomes can serve as input for a database).

- In case of international trade, incidents may be prevented by exporting nations by implementing quality systems, rigorous regulatory requirements, and checks of consignments destined for export. In some of the cases described, as well as in other reported cases (such as antibiotics in shrimps imported from Asia), this was actually done after the incident had already occurred

Whereas food safety is within the mandate of EFSA and the EU's national food authorities, it is likely that in order to put these recommendations into practice, cooperation with the private sector as well as with foreign nations should be pursued.

The abovementioned recommendations focus on the factors within the food manufacture chain that may give rise to the occurrence of risks. It is realized, though, that this chain is embedded within a wider environment comprising different sectors that may each influence the occurrence of risks to the safety of food and feed. Interestingly, the PeriApt project has developed a model for the early identification of emerging risks by definition of influential sectors in which particular changes may already highlight the potential for new or increased risks within the food manufacture chain. This model has been further developed by the EMRISK project, which adapted the definition of influential sectors, and identified a set of indicators within the influential sectors, of which changes may give rise to signals for potential risks. EMRISK also devises a procedure in which expert panels interpret the outcomes of automated Internet searches for information on potential risks (EFSA, 2006a). EFSA, which had commissioned the EMRISK project, has considered EMRISK's recommendations and has acknowledged the importance of expert judgment in the process of identification of emerging risks and the availability of expert networks that can be contacted for this purpose (EFSA, 2006b). In more general terms, these outcomes show that against the background of environmental factors ultimately affecting health, the importance of inter-sectoral and multidisciplinary collaborations for the early identification of food and feed safety risks is becoming increasingly acknowledged.

In addition, there are a number of human and policy factors that apparently have played a role in the featured case studies, such as the behavior of producers and traders within the manufacture chain, perception of risks by consumers, and motives underlying decisions taken by policy makers. Therefore, the ethics surrounding the issue of food safety are an important factor to be considered. In fact, the striving towards food safety and its global harmonization partly derives from the moral obligation to protect and improve the health of humans and animals. This would align with the ethical principles of non-maleficence, reduction of harm, and creation of benefits. For food safety policy development, it would therefore be useful to gain insight into the ethical values held by the various stakeholders involved in food and agriculture. A tool that has been developed for this purpose is the Ethical Matrix, which helps to visualize the ethical considerations shared by stakeholders that are consulted as participants of dedicated ethical workshops (Kaiser et al., 2007). A number of studies also indicate that the perception of risks may vary between policy makers, scientists, and consumers. This difference in perceptions is also exemplified by the incident involving the occurrence of organochlorine residues in salmon described above. For example, risks may be amplified in public perception if associated with fear, or may be negated or optimistically viewed if consumers' lifestyles are implicated (Verbeke et al., 2007).

In conclusion, the case studies of past occurrences of biochemical and chemical risks to food safety described above have provided insights as to how similar incidents may be prevented in future, amongst others by detection at an early stage of development. Whilst the focus has been on the European situation, the conclusions and recommendation are also applicable to non-European settings.

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#### **5. Conflict of interest statement**

The authors are professionals employed by RIKILT - Institute of Food Safety and by IMARES – the Institute for Marine Resources and Ecosystem Studies, both part of Wageningen University and Research Center, and as such do not have any interests that may conflict with the contents of the article above.

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ACCEPTED MANUSCRIPT

**Tables****Table 1 Some illustrative examples of recently reported incidents in 2004-08 involving commingling or cultivation of crops with GM varieties unauthorized in country of reporting\***

<i>Crop</i>	<i>Unauthorized GMO</i>	<i>Country</i>
Cotton	Herbicide resistant	Brazil
Oilseed rape	Herbicide resistant	Australia
Maize	Insect resistant	US
Maize	Herbicide and insect resistant	Chile
Papaya	Virus resistant	Germany
Rice	Insect resistant	China
Rice	Herbicide resistant	US
Soya	Herbicide resistant	Hungary

\* taken from the "GM contamination register" website (<http://www.gmcontaminationregister.org>)