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Inventory of possible emerging hazards to food safety and an analysis of critical factors

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MANAGEMENT SUMMARY

Various recent incidents around food safety issues have led to the establishment of the European Food Safety Authority and its national counterparts in the member states of the European Union. Besides these measures, it is desirable to identify food risks while they are still emerging, *i.e.* when they still are hazards.

This report provides the outcomes of subproject 2.1 of the WUR-LNV project BO-08-002, which aims at supporting governmental policies towards early identification of emerging food risks. In particular, this subproject focuses on issues that are still ongoing and have the potential to cause new problems. This contrasts somewhat with the issues discussed in subproject 1.1, where the focus is more on "learning from the past." Another difference is that 1.1 focuses on the pro-activeness of risk management measures, while 2.1 also pays attention to the scientific data underlying these issues and to their international dimensions. The criteria for selection of the issues in subproject 2.1 have been i) the inclusion of both chemical and microbiological hazards in products of both vegetable and animal origin, ii) the relationship with either high- or low-input agriculture, iii) the availability of expertise and sufficient documentation on the background of the issue and iv) any risk management measures that might have already been taken. Based on the review of the issues selected, recommendations have been formulated on how to identify and prevent these and similar issues in an early stage of their development.

This report considers the following two issues:

1. The use of antibiotics in intensive aquaculture and shrimp farming. The example of the use of chloramphenicol and nitrofuran antibiotics, which led to recent trade issues on farmed shrimps containing residues of these antibiotics, is reviewed. Because of these residues, the import of the shrimps into the European Union (EU) had been blocked. The use of these antibiotics relates to disease pressure associated with intensive aquaculture. While these antibiotics are known hazards, it is envisaged that identical and alternative antibiotics in aquaculture products from the same and other geographical areas may form an emerging hazard; and
2. Pesticides of natural origin, such as chemical products (*e.g.* a mixture of chemical substances obtained by extraction of a plant) and microbiological products (*e.g.* insecticidal spores of the soil bacterium *Bacillus thuringiensis*). Two cases are considered in more detail, *i.e.* rotenone and its reported relationship with parkinsonism, and neem tree extract, which is considered safe, but of which the composition may change due to altered extraction methods.

For both issues, it is concluded that besides any food safety aspects of residues of these substances in food and feed products, absence of internationally harmonized regulations on such residues may lead to more stringent risk management measures taken by importing nations.

The recommendations put forward on the issue of antibiotics in aquaculture products are

1a) to organize farmer education and quality control systems;

1b) to improve regulations and international harmonization;

1c) based on indicators identified in the report, to pro-actively search for antibiotics and alternatives that might be used in aquaculture and in this way try to avoid that they might become a concern in future; and

1d) to carry out a more general pro-active survey on the use of chemicals in aquaculture, based on the indicators identified.

The recommendations on the issue of pesticides of natural origin include:

2a) to consider that adapted extraction methods may introduce new hazards to botanical products used as pesticides that are currently considered as safe;

2b) to follow an equal regulatory risk assessment approach towards natural and synthetic pesticides if they are expected to be neurotoxic;

2c) to take into account that living organisms used as biopesticides may constitute new hazards because of their ability to multiply after application.

The review of these issues also shows that progress in science may reveal previously unsuspected hazards including previously unknown associations between certain agents and disease, such as for pesticides and parkinsonism, and also for the presence of low levels of antibiotics in food, such as for chloramphenicol and nitrofurans in shrimp. Indicators emanating from this and other studies may be used for the pro-active identification of emerging issues that deserve attention from risk managers with regard to possible interventions to prevent them developing into real risks.

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1 INTRODUCTION

Recently, a number of incidents concerning food and feed safety occurred, such as the occurrence of dioxins in animal feed, bovine spongiform encephalopathy (BSE), nitrofuran residues in poultry and seafood, and foot and mouth diseases. In order to cope with similar incidents in a coordinated fashion in future, the Dutch national Food and Consumer Product Authority and its counterparts at the regional level of the EU and at the national level of other member states have been established.

In retrospect, the question may be put forward if and how the said incidents might have been prevented from occurring at an early stage. Actually, this project focuses on methods for the early identification of emerging hazards. Within subproject 1.1, for example, a number of case studies have been investigated. These cases concerned incidents of the past, where sufficient data were available for the chronological reconstruction of the scientific and non-scientific features of these cases. These cases include for example the BSE-crisis and the recent contamination of cow's milk with dioxins derived from animal feed containing potato processing by-products contaminated with dioxin-containing clay. This way, it might be learned what went "wrong" and how similar incidents can be prevented in future. Among others, risk communication and risk management issues are important features in the case studies of subproject 1.1.

Within this subproject, *i.e.* 2.1, two activities were envisioned in the original project proposal submitted to the Dutch national Food and Consumer Product Authority.

First, an inventory was to be made of the existing initiatives that aim to identify emerging food risks in an early stage. In addition, the methodologies used by these initiatives for identification were to be reviewed. During the course of the project, it became clear, however, that this activity was also undertaken by various parallel initiatives, such as the EFSA-sponsored EMRISK project led by the Dutch national Food and Consumer Product Authority.

Within EMRISK, an inventory is made, among others, of the EU-sponsored research projects of the Fourth, Fifth, and Sixth Framework Program that directly or indirectly address the issue of emerging hazards to food safety. In addition, it identifies the presence of networks that can be exploited for consultation on emerging issues. Another parallel initiative is developed within the SAFE FOODS project's work package 2. In this case, the tasks of the work package include the setting up of a framework to identify emerging food risks. This frame work will be based on the experiences of the participants in the areas of microbiological and chemical risks, including also mycotoxins, with a particular view on risks associated specifically with agricultural practices requiring high or low inputs. These two projects, EMRISK and SAFE FOODS, were still ongoing at the time of writing, and some researchers of RIKILT within these projects also participate in the current BOP project. It was therefore decided to avoid duplication and to limit the extent of the current review of initiatives in the area of emerging food safety hazards, while awaiting the further outcomes of the parallel initiatives.

Secondly, a number of case studies on emerging chemical and/or microbiological hazards were to be reviewed. These cases would differ from those of subproject 1.1 in that they would focus on emerging hazards that are "contemporary," meaning that they are or may still become real risks. In addition, cases were sought for that would include both vegetable and animal production chains, chemical and microbiological hazards, and association with either high- or low-input agricultural practices. Recent, well-documented incidences involving the safety of the selected items should provide a window on the management issues, which could facilitate the formulation of recommendations for the management of these and similar cases in future. In addition, sufficient knowledge should be available on the scientific background, such as mechanisms underlying toxicity, and also on the regulatory environment of the selected items. The case studies should lead to the formulation of indicators that may be used to screen for the occurrence and development of these and similar hazards. Based on these criteria as well as the ones used in the selection procedure for cases in the parallel subproject 1.1 (type of emergence, timeliness and mode of detection during incident, involvement of other sectors than food production, relevance to human health, international dimension, impact), discussions among a select number of experts within the participants institutes thus led to the selection of the three case studies. These studies differ from those in 1.1 in that, for example, the latter puts more emphasis on the role of overarching conclusions on the pro-activeness of management for the prevention of the cases considered in hindsight. The studies in 2.1 on the other hand also devote a great deal of attention to the scientific issues underlying the mechanisms of hazard formation and to the international dimensions, such as differences in national regulatory requirements. There are a number of links, however, between the studies, such as the cross-cutting issues in seafood, environmental contaminants, and intensive production methods. The three chosen case studies were the following:

- **Use of antibiotics in intensive aquaculture of shrimp and fish.** The practice of aquaculture is growing worldwide and various international and national authorities actually support this development. However, these intensive farming practices may have the undesired side-effect of increased disease pressure. In order to mitigate the spread of disease among the cultured fish or shrimp, and the resulting economic loss, prohibited antibiotics have been used in the past. Recent incidents with the presence of residues of known, prohibited antibiotics in shrimp, such as chloramphenicol, can serve as a model for the application of novel, lesser known antibiotics. This case is an example of a chemical hazard in intensive animal production.
- **Pesticides of natural origin, which may include both chemical and microbiological products.** There is no harmonized international approach yet towards the regulatory requirements for these products. Actually, the regulatory requirements may in some countries be more "relaxed" than for conventional pesticides. In addition, a rise in the use of this type of pesticides may be anticipated given the current EU policy to discourage use of synthetic pesticides and to promote organic agriculture, for which these pesticides may be the only option to control pests or diseases. A well-known historic example is the insecticide rotenone, which is made from an extract of plant roots. In a rat experiment that received media attention, it was shown that this pesticide was able to induce effects resembling Parkinson disease. Another example is neem tree products. A number of these products have a history of safe use. However, there is uncertainty about the nature of substances that are responsible for the noxious effects of some types of extract, which may indicate an emerging hazard for new uses or products of neem tree. This case study is an example of both chemical and microbiological risks. While pesticides may be used on agricultural crops, there are also applications on animals, such as disinfectant or piscicide, *i.e.* a chemical that is used to control fish. Pesticides of natural origin are currently predominantly linked with low-input and/or organic agriculture, but are likely to become increasingly used in conventional agriculture as well. In addition, new insights gained with these pesticides may also be relevant for synthetic pesticides as well.
- **Toxoplasmosis in organic and free ranging swine husbandry in comparison with conventional husbandry.** *Toxoplasma* is a parasite, which is a well-known pathogen that frequently occurred several decades ago in swine production systems, but has dropped to almost zero-level due to the intensification of swine husbandry. It can be transmitted through the consumption of insufficiently heated meat and infections can lead to abortions, neurological impairment of newborns, ocular damage, and encephalitis in immunocompromised individuals.

> A recent study carried out by Dutch scientists reported the occurrence of *Toxoplasma* on several organic and free range swine farms. It was reasoned that a number of factors might have contributed to this, including the use of cats, which may carry *Toxoplasma* with them, to combat rodents; and free-ranging of domestic animals that can come into contact with cat defecations. In this case, the occurrence of *Toxoplasma* would constitute a "re-emerging hazard." Since cats appear to play a central role in the dissemination of the hazard, this has to be put into perspective with regard to other related hazards, such as the risk of transmission to pet owners holding cats. This case is an example of a "re-emerging" microbiological hazard in animal production, particularly with organic or other free-ranging production systems.

These case studies were to consist of literature reviews, whose first authors are experts in the pertinent fields of food safety research.

As regards the case study on *Toxoplasma*, it was decided to put this part to rest until a panel that had been installed by the Dutch national food authority on the same subject would have finalized its own review and recommendations. By the time of this decision, a draft report on *Toxoplasma* had already been prepared by Prof A. Kijlstra. This report is enclosed as a separate, confidential document.

Based upon the outcomes of the particular case studies, some generalized conclusions regarding indicators for the early identification of emerging hazards, as well as recommendations on measures to mitigate these types of hazards, will be provided.

In addition, a third feature to be explored within this subproject was the possible relationship between food safety risks and imports. Initially, a theoretical framework was to be formed, based upon a review of the literature on international trade and related food safety issues from an economic point of view. This will take into the various actors that are active within the international food production and trade chains, and the forces that are acting upon the market. Possible indicators that are identified in this research will be further investigated in the second phase of the study, which may involve the opinions of experts, the results of border inspections, and the outcomes of parallel initiatives, such as the SAFE FOODS project. This part of the subproject is carried out by the Institute for Agricultural Economics. The outcomes of this study will be reported separately.

2 EXISTING INITIATIVES TO IDENTIFY EMERGING RISKS

A general, non-exhaustive overview of operating systems and initiatives that identify emerging food safety hazards, or these hazards as part of activities in a broader sense, is provided in *Table 1*. Given that other projects, such as EMRISK and SAFE FOODS are also studying these initiatives, this section for the time being does not go further into detail, which may be done so in future activities of this subproject, if this is still needed.

Table 1: General overview of initiatives for the identification of emerging hazards to food safety specifically or as part of activities with a broader scope

Initiative	*Description*
OIE - World Animal Health Information System (international, http://www.oie.int/eng/info/en_info.htm)	Certain animal diseases that occur in member states, including zoonoses and/or emerging diseases, have to be notified through this system. This may include pathogens that are relevant to food safety.
WHO- Global Outbreak Alert and Response Network and Global Public Health Intelligence Network (GOARN and GPHIN; international; http://www.who.int/csr/outbreaknetwork/en/; http://www.who.int/csr/alertresponse/en/)	Human diseases that may become of international importance should be notified through GOARN, after which WHO may decide to take appropriate measures. In addition, through GPHIN, media sources and other informal data are screened for indications of potential epidemics etc., including issues that are related to food safety
EU Scientific Committee on Emerging and Newly-Identified Health Risks (SCENIHR; EU; http://europa.eu.int/comm/health/ph_risk/committees/04_scenihr/04_scenihr_en.htm)	The field of work of SCENIHR not only covers food, but also other issues, such as new technologies
DEFRA - Horizon Scanning (UK, http://www.escience.defra.gov.uk/horizonscanning)	This project entails "foresight" of potential issues by exploring demographic trends and prospecting in non-traditional scientific areas. Ideas for research can be submitted. Issues cover the remit of DEFRA, including food issues.
USDA-APHIS Center for Emerging Issues (USA; http://www.aphis.usda.gov/vs/ceah/cei/index.htm)	This center identifies issues that may affect animal diseases or trade in animal products from the USA. Topics are mainly microbiological, but occasionally also chemical cases are included. Foreign developments may be followed that have the potential to become problems for US animal production and exports

Initiative	*Description*
EPA ORD - Research on "environmental futures," including "emerging pollutants" (USA, http://epa.gov/osp/futures/aboutgoal.htm)	Given that there are thousands of industrially produced chemicals that have not been fully assessed for their safety, the EPA ORD aims to identify "emerging" pollutants whose occurrence in the environment has been altered or previously unknown, or whose toxicity has previously not been known. Whereas this pertains to emerging pollutants such as medicines or personal care products, there may be links to food safety in some cases

Abbreviations: APHIS, Animal and Plant Health Inspection Service; DEFRA, Department for Environment, Food and Rural Affairs; EPA, Environmental Protection Agency; OIE, Office International des Epizooties; ORD, Office of Research and Development; USDA, US Department of Agriculture; WHO, World Health Organization

3 CASE STUDIES FOR THE DEFINITION OF INDICATORS AND APPROACHES TOWARDS EARLY IDENTIFICATION OF EMERGING FOOD SAFETY RISKS

The two case studies on antibiotics in culture shrimp and pesticides of natural origin are provided in annex to this report. While they are discussed more in detail within the pertinent sections (*i.e.* appendix 1 on antibiotics in cultured shrimp and appendix 2 on pesticides of natural origin), some generalized conclusions can be inferred from these studies.

3.1 Antibiotics in shrimp

In the case of antibiotics in shrimp, there have been previous incidents involving the occurrence of the antibiotics chloramphenicol and nitrofurans in shrimps imported from Asian countries into the EU. A number of factors appeared to have played a role in the development of this case into an incident. For example, there were no internationally harmonized health criteria for these antibiotics, such as for other compounds through Codex alimentarius, because of a lack of data on certain toxic effects. The EU therefore initially followed a "zero tolerance" policy. In addition, detection methods for antibiotic residues in edible animal products were continually being improving, with detection thresholds being lowered, hence increasing the likelihood of detection of trace residues.

In addition, these antibiotics were readily available in the exporting countries where no regulations and/or controls on these antibiotics existed. The use of these antibiotics may have further been promoted by the increased disease pressure associated with intensive shrimp farming practices. In addition, absence of resistance among shrimp pathogens, such as *Vibrio* spp., towards these particular antibiotics might have contributed to their use. Besides these factors, it should be taken into account that in some cases, illegal use of antibiotics may also occur.

Following the detection of CAP in shrimps, the EU intensified its border controls or, in some cases, barred imports of shrimp from various Asian countries. These measures were suspended after a period in which the exporting nations had established appropriate controls on consignments of shrimp destined for export and quality systems to ensure that no antibiotics were used in aquaculture. In addition, the "zero tolerance" approach was replaced by the definition of "minimally required performance levels" for control laboratories.

In *Table 2*, the indicators identified in this study are summarized. In addition, potential synergies between these indicators, as well as information sources are described more in detail within the case study report (see appendix 1).

Table 2: Indicators for emerging hazards associated with the occurrence of antibiotic residues in aquaculture products

Indicator	*Comment*
Lack of internationally harmonized legislation and quality assurance	The use of antibiotics may be allowed in the exporting nation, while being prohibited by importing nations. In addition, there may be lack of control on the use of antibiotics in primary production.
Zero tolerance and background presence of antibiotics	Due to lack of toxicity data, some antibiotics are not allowed at all. However, with analytical methods becoming more sensitive, there is an increased likelihood of detection, especially for compounds that occur at low background levels
Illegal use or easy access to antibiotics	This is associated with a number of factors, such as the degree of enforcement of prohibition of antibiotics in products or the lack of their prohibition, as well as the cost-effective production of antibiotics in some countries
Sharp increase in production and trade of farmed fish or shrimp	Usually in the years following initial rapid increase of production, disease pressure increases, which in turn may lead to antibiotic use, especially if financial resources do not allow for sustainable alternatives

In addition, the study puts forward a number of recommendations, such as:

- Farmer education and introduction of quality control systems. For example, farmers should be made aware of the issues surrounding the use of antibiotics, such as antibiotic resistance. In addition, the principles of quality systems like Hazard Analysis and Critical Control Point (HACCP) should be implemented, since these enable a better control of the use of antibiotics
- Improvement of regulations and their international harmonization. For example, a registration system for antibiotics would at least provide insight into the distribution and uses of these antibiotics. In addition, the formulation of "minimally required performance levels" (MRPLs) has in the past helped to avoid problems arising from a "zero tolerance" policy by serving as the lowest “non-zero” levels that can be reliably measured, although MRPLs may be stringent too. The regulatory requirements of HACCP in shrimp production and certification of consignments for export have proven beneficial in the past. However, these requirements were implemented post hoc, *i.e.* after the EU has imposed measures on exports. Therefore a pro-active ex ante approach might help avoiding new incidents from occurring.
- A pro-active search into antibiotics and alternatives that may be used in aquaculture and that would be of concern if they were present as residues in aquaculture products. This search may take advantage of the indicators formulated above.
- A more general pro-active search on chemical use in aquaculture, based upon the indicators above.

3.2 Natural pesticides

As regards natural pesticides, these may consist of chemicals, such as extracts from botanical species, or biological products, such as preparations containing live bacteria. Actually, this type of pesticides may in some cases be the only pest management solution for organic farmers. This is because they are allowed only to use a limited number of these pesticides under regulations on organic farming in the EU and internationally. Whereas the nature of these pesticides confers to them an environmentally friendly image, a number of safety issues may still pertain to them. For example, natural products may contain toxic substances. Actually, the data on the precise nature of the chemical constituents of some source materials, such as plants, used for pesticide production may be incomplete. Microbiological products have the ability to reproduce and multiply after application, giving rise to other behavior than known for synthetic chemical substances.

Two cases are highlighted in the study on natural pesticides, *i.e.* i) the neem tree, which is a plant grown worldwide, the products of which are appreciated not only for their pesticidal action, but also as alternative medicine; and ii) rotenone, a pesticide made from plant root extracts, which recently received media attention given a possible link with Parkinsonism (see also appendix 2). Actually, there have been no incidents regarding food safety in particular with these two natural pesticides, unlike the case study with antibiotics. Therefore, this study is more concise than that on the antibiotics in cultured shrimp.

In the case of neem tree products, it is noted that there has been a history of safe use of a variety of neem tree products, such as aqueous extracts and neem oil, as insecticides. In theory, residues of neem tree products may remain on food products. However, apart from some anecdotal accounts of incidents involving intake of high levels of residues, data so far do not give rise to concern over the potential harmful effects derived from these residues in general. Besides the products that are commonly used, non-aqueous extracts of neem are known to be toxic, while no information is available yet on the precise nature of their toxic constituents. This uncertainty and lack of information may therefore give rise to an emerging hazard if new extraction methodologies are to be applied to neem, such that the unknown toxic compounds might enter pesticides and other neem products used in agricultural production.

With regard to rotenone, the possible link with Parkinson's disease was brought to the attention by media reports on a rat study in which high intravenous doses caused lesions in brains and symptoms characteristic of Parkinson's. Actually, the researchers had chosen to investigate rotenone because it had the same mechanism of action as MPTP, a well-known neurotoxin derived from a contamination of synthetic heroin. Whereas the route of administration in the rat study may not be representative of human exposure either through inhalation or oral consumption, this study still offers some useful perspectives on emerging hazards of pesticides in general.

For example, there is increasing evidence from epidemiological and laboratory studies that there is a link between Parkinson's disease and previous exposure to pesticides and other environmental factors. Whereas the epidemiological studies cannot pinpoint specific chemicals as causes, it may noteworthy that for instance the herbicide paraquat has a structure that is similar to MPTP and also shows neurotoxicity in brains of experimental animals. Synthetic pesticides may be more persistent in the environment and more likely to occur as residues in food and be taken up from the intestines than rotenone, which may indicate an emerging hazard. The emergent nature of this hazard is actually based on the developments in scientific insights into the action of "old" chemicals.

Based upon the items reviewed in this case study, as well as the recommendations it puts forward in this case study, a number of indicators of emerging hazards associated with pesticides of natural origin and pesticides in general can be identified, which are listed in *Table 3*.

Table 3: Indicators for emerging hazards associated with the use of pesticides of natural origin, as well as pesticides in general

Indicator	*Comment*
New methods for producing pesticides of natural origin, *e.g.* change in extraction method	The source materials are complex and may be incompletely characterized with regard to potential toxins that may become part of the newly processed pesticide
Presence of living organisms within a pesticide	Unlike chemical compounds, organisms can multiply and/or reproduce themselves after their release
Lack of internationally harmonized regulations on pesticides of natural origin and pesticides in general	Pesticides of natural origin are subdue to "relaxed" regulations in some countries, while in others (*e.g.* EU, Central America), to the same rigor as for conventional pesticides. This situation may be further exacerbated by additional national regulations on which pesticides are allowed in organic agriculture, or voluntary agreements on conventional pesticides.
Incomplete knowledge on the nature of toxic components of source materials used for production of pesticides, such as plants	As in the case of neem tree, one type of extract is known to be toxic by experience. However, the identity of the toxic compound is not known yet.
Incomplete knowledge on the etiology of affections that may have their sources in the environment or food, such as Parkinson's disease	As in the case of Parkinson's disease, evidence is accumulating that there is a link with previous exposure to pesticides and other environmental factors
Similarity of chemical structures, biochemical mode-of-actions, or biological features to those of a known hazardous substance or organism	The pesticide rotenone has the same mechanism as the neurotoxin MPTP, while paraquat has got a similar structure.

In addition, it appears that some of these indicators also have links with Good Agricultural Practice as regards the use of these pesticides, including the application rate and frequency of use, as well as Good Manufacturing Practices, including the batch quality of natural products.

4 OVERALL CONCLUSIONS

As divers as the case studies may seem, some general inferences can be made regarding the factors that contribute to the emergence of hazards and their development into real risks.

For example, the lack of internationally harmonized regulations may not constitute a hazard *per se*, but can aggravate a problem in terms of economic cost and political dimensions if an importing nation has to bar unfit consignments from import at the port of entry. Furthermore, both for natural pesticides and antibiotics, the restrained financial resources in the country of production may give further impetus to their usage.

Furthermore, progress in science may contribute to the emergence of hazards that previously have been unknown. For example, previously unknown relationships between certain factors, such as chemical compounds and micro-organisms on one hand, and diseases on the other have been discovered. In addition, advances in analytical science may facilitate detection of traces of contaminants at infinitesimal levels in food products. Actually, in these cases, it is not the hazard that is novel, but the awareness of it. In addition, pro-active searches may be performed based on the indicators identified in these studies, in order to identify products and hazards that would need further scrutiny in risk management.

In conclusion, subproject 2.1 has studied various cases of currently emerging hazards and formulated a number of indicators that are useful for screening, as well as recommendations on how to mitigate emerging hazards similar as the ones having been studied. In combination with subproject 1.1, these outcomes may provide a basis for further elaboration into a broader and more general framework for early identification of emerging hazards to food safety.

5 ACKNOWLEDGEMENT

The contribution of Prof A. Kijlstra on the issue of *Toxoplasma* in livestock is gratefully acknowledged. The national Food and Consumer Product Safety Authority has contributed to the idea for investigating the topic chosen for the project. In addition, the authors wish to thank the Ministry of Agriculture, Nature, and Food Quality, as well as the VWA for their generous financial support to project BO-08-002 and for their directions and feedback in the course of the project.

Appendix 1: Case study on antibiotics in cultured shrimp

M. Poelman, G.A. Kleter, H.J.P. Marvin

Introduction

The use of antibiotics (chloramphenicol and nitrofurans) in shrimp farming has been chosen as an example of an emerging risk, since it has had severe consequences for the industry. In addition, the outcomes of this case study may help to identify possibilities for earlier detection of similar problems in seafood produced through aquaculture in future. For the choice of this case study, the following deliberations were considered particularly relevant:

- The problems which are associated with the use of antibiotics in shrimp production are also expected in other aquacultural species, since aquaculture is still an increasing on a global scale and similar diseases are common in many species;
- Especially in developing countries, disease-preventing chemicals are usually a cheap and very effective tool to increase the production;
- Sufficient data is available on measures that have been taken upon the emergence of the food safety problem making a complete evaluation possible;
- The food safety problems encountered in shrimp production have had significant economic and political impact both in the exporting Asian countries and in the EU;
- The use of antibiotics and the attention it received in the European media influenced consumer perception and affected trade potential in a negative way;
- The discovery of antibiotics in shrimp has probably not been the result of any rapid increase in antibiotic use, but is due to, at least in part, the improvement of analytical methods. The use of LC-MS-MS methodology made chemical analyses much more sensitive than before, allowing detection of lower amounts. This phenomenon has also been observed in other instances in which the application of new / novel antibiotics in the production of seafood was discovered. In addition, discovery of the presence of antibiotic residues commonly results in a increase of control measures, which also increases the discovery rate;
- Finally, the case on shrimp antibiotics can be considered an interesting example because not only does it pertain to the use of antibiotics as such, but also to a special class of chemical compounds without internationally harmonized acceptable daily intake or maximum residue level (ADI/MRL).

The abovementioned arguments subscribe to the lessons that can be learned by studying this case in more detail. Furthermore, it is apparent that trends are expected within and outside the seafood production chain that favor the emergence of new food safety risks.

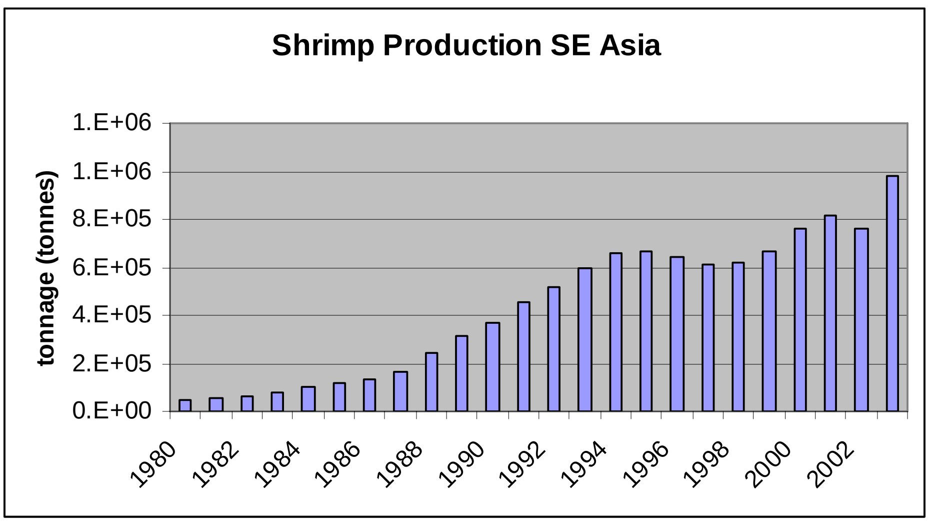
The urgency of this topic is also acknowledged on a political level as illustrated by the bilateral projects between the Dutch Ministry of Agriculture Nature and Food Quality (LNV) and several Asian shrimp-exporting countries. In these projects, possible alternatives to the use of antibiotics are investigated. This is done via the Partnership on Market Access, which was initiated at the World Summit on Sustainable Development (WSSD) in Johannesburg in 2002 (LNV, 2005).

Besides this collaboration, the European Commission has launched initiatives on intensification of disease monitoring and prevention in fish cultivation systems, in addition to new, harmonized legislation on aquaculture (European Commission, 2005).

The problem in short

During the last decade, shrimp farming in South East Asia has increased rapidly, in particular for the production of Penaeus, Akiami paste, Metapenaeus, and Whiteleg shrimps, as well as Banana, Giant tiger, and Indian white prawns (*Figure 1*; Josueit, 2004). The increasing demands were resolved by the intensification of shrimp aquaculture, which was stimulated by the industry, governments, and international organizations (*e.g.* Lebel et al., 2002). Also, the intensification concurred with increasing use of antibacterial components, *i.e.* antibiotics, in order to prevent losses due to diseases (*e.g.* Lebel et al., 2002). The impact of the industry on local economies is great, since in many regions, the economy depends on shrimp production on an economic base (De Farias Costa and Sampaio, 2004). Beyond the regional level, the shrimp industry is also important for many Asian countries and globally operating companies that have intensive trade relationships with these countries. Issues that are associated with shrimp productions thus may have a negative or positive impact on trade relationships.

Figure 1: Shrimp production South East Asia from 1975-2003. Species included are: Banana, Giant tiger, and Indian white prawns, as well as Akiami, Metapenaeus, Penaeus, and Whiteleg shrimps (Source: FAO, 2005)



The major disease-causing agents in shrimp cultivation are *Vibrio* spp. and viral agents. Attempts have been made to prevent the occurrence of these pathogens, initially by the use of antibiotics and later by the use of prebiotic and probiotic preparations. Whereas viral diseases could not be eliminated by antibiotics, the increased use of antibiotics sustained due to lack of education and knowledge. In addition, the heavy antibiotic regimes applied have resulted in an increase of antibiotic resistant bacterial strains in the environment and human pathogens, and may therefore also become a food safety concern (Alderman and Hastings, 1998; Holmstrom et al., 2003). After the first detection of chloramphenicol in shrimp products, the European Commission and foreign agencies, such as the American FDA, increased their checks of imported shrimp for antibiotics. Exports that mainly went to the EU decreased, probably due to imports being barred or avoidance of the severe impact and financial risks of destruction of the exported product.

Risk of exposure to chemical/biological agents

In the case of the cultured seafood products being contaminated with antibiotic residues, the route of exposure to the hazard will be oral, *i.e.* through consumption of aquaculture products. This means that the product may have undergone processing prior to consumption and that the gastro-intestinal bio-availability of residues from the product, as well as their possible metabolism by the intestinal microflora, can have an impact on the effects on the consumer.

For chloramphenicol (CAP), other routes of exposure are through medicinal use (*e.g.* typhoid fever) and by application of ophthalmic solutions (*e.g.* ocular ointments).

How the product is produced: production chain

Antibiotics in intensive farming may be used for preventive purposes. In shrimp and fish farming, antibiotics may be administered through mixing of antibiotics with fish feed. In addition, some unconfirmed accounts exist of the use of antibiotics applied as a powder on top of fresh catchments of shrimp or fish in cases of lacking refrigerating capacity.

Pathogenic bacteria, such as *Vibrio* pathogens, are naturally present in seawater in which shrimp/fish is reared. Resistance to CAP in *Vibrio* and some other shrimp pathogens is generally absent or very low (*e.g.* Otta et al., 2001), which renders the use of CAP against these pathogens attractive. For nitrofurans, reports vary from broad-scale sensitivity to resistance amongst *Vibrio*, depending on the source, *i.e.* clinical versus environmental (*e.g.* Roque et al., 2001). Whereas the use of CAP, nitrofurans, and some other antibiotics used in seafood farming may have been prohibited within the EU, the legislation of some seafood-exporting nations previously allowed the use of antibiotics. In fact, some anecdotal accounts tell of small shops for fish farmers where these antibiotics could be obtained.

Shrimps destined for exports are commonly checked by governmental export agencies before being shipped overseas. Before being exported, shrimps may be washed, dried, decapitated, peeled, deveined, and cooked, which are considered to be steps that add value to the product. Peeling can be done manually, mechanically, or enzymatically. Deveining, *i.e.* removal of gastrointestinal tract, is labor-intensive and may therefore be economic for larger and more expensive shrimp species. Many of the tropical peeled shrimp imported into the EU have been peeled raw, but not deveined ("peeled undeveined"), and, after freezing and transport, are to be processed further, *i.e.* by cooking. The processing stages may also provide for potential points of entry for pathogens, which can be controlled by HACCP. Shrimps may also be further processed into shrimp meal (*e.g.* "kroepoek," shrimp crackers) and chitin (by-product of peelings). Processing of shrimp for EU markets is usually supported by HACCP principles, which minimizes the risk of post harvest contamination.

Toxicity: intake and symptoms

Both CAP and nitrofurans are rapidly absorbed from the gastrointestinal tract after oral uptake (*e.g.* INCHEM, 1997; JECFA, 1993ab, 1994, 2004). CAP is also metabolized by the intestinal microflora to various metabolites, including dehydro-chloramphenicol, nitroso-chloramphenicol, and nitrosophenyl-chloramphenicol. After uptake, these antibiotics are distributed to all organs. The main route of excretion of CAP is through the urine, *e.g.* as glucuronides.

After intake of therapeutic doses, CAP may cause irreversible aplastic anemia (*e.g.* depletion of blood cells), reversible depression of bone marrow cell production, and, eventually, leukemia. Especially children appear to be sensitive to these effects. Aplastic anemia rarely occurs in the populations and in many cases has fatal consequences. No association could be established between the use of CAP for ocular treatment, after which the systemic uptake appears to be limited, and the occurrence of aplastic anemia. In addition, the Codex Committee (JECFA, 1994) concluded that if the exposure through food to CAP equals that from ophtalmic use of CAP, no demonstrable alteration of incidence is expected.

Nitrofurans that have been evaluated by Codex include furazolidone, nitrofurantoin, and nitrofurazone (nitrofural). Besides carcinogenicity in animals and/or genotoxicity/mutagenicity, acute effects of medicinal doses include lung toxicity of nitrofurantoin, for example. In addition, it was noted that additional data would be needed on the chemically bound residues of metabolites of furazolidone in tissues (JECFA, 1993ab; INCHEM, 1997).
Neither an acceptable daily intake (ADI) nor a maximum residue level (MRL) has been defined by Codex, since no sufficient data on CAP and nitrofurans are available for carcinogenicity.
For veterinary drugs without an ADI/MRL like CAP and nitrofurans, it is problematic to extrapolate these effects to low-dose intakes, such as encountered by food consumption. Actually, a risk assessment of the occurrence of CAP in imported shrimps by the Dutch Institute for Public Health and Environment estimated that the intake of CAP by consumers from food would be by a factor of 150 million till 735 million lower than to therapeutic doses (Hanekamp and Kwakman, 2004).

How it becomes a problem in the production chain
A recent workshop convened by the FAO/WHO in 2004 addressed the issue of these drugs without ADI/MRL and highlighted in retrospective the factors that had led to the crisis regarding antibiotic residues in seafood in 2001-2002 (FAO/WHO, 2004). For veterinary drugs like CAP and nitrofurans, no ADI/MRL exists, as explained above. Therefore, some nations, including the European Union, showed "zero tolerance" to these compounds. This means that any contamination of foods by residues of these veterinary drugs above the limit of detection will be considered contaminated. On the other hand, increasingly sensitive analytical methods are developed that can measure minimal amounts of antibiotics (FAO/WHO, 2004).
In addition, for nitrofurans, the focus of the regulatory framework initially was on the intact molecules, which were considered to be the toxic agent. Conversely, attention currently is also paid to the metabolites of these antibiotics, which may be toxic as well as in some cases and serve as analytical markers (BgVV, 2002).
Recently the EU defined "minimally required performance levels (MRPLs)" for compounds without ADI/MRL, *i.e.* the level where they can still be reliably measured at from a statistical point of view (Decision 2002/657/EC). In practice, this means that MRPLs constitute the lowest levels that are considered “non-zero” in the enforcement of “zero tolerance.” Later, these MRPLs were defined as action levels for residues of certain chemical compounds (Decision 2005/34/EC). Consignments of products with levels of these compounds exceeding the MRPL should either be destroyed (possibly after recall) or shipped to a third country outside Europe after approval by the latter. For example, MRPLs in aquaculture products amount to 0.3 ppb for chloramphenicol and 1 ppb for nitrofurans (furazolidon, furaltadon, nitrofurantoin, nitrofurazon).
In addition, before the recent incidents, legislation in countries of origin allowed the use of forbidden antibiotics. There have also been accounts of illegal use. At least, it may be clear that there was no harmonization, even not among Western countries that imposed different levels-of-detection for the same antibiotic in the initial phases of the incidents prior to EU-harmonized measures. The problem was enhanced by the lack of guidance by Codex Alimentarius, since there was no scope for residues without MRL.

Some authors postulate that there is a background environmental presence of antibiotics due to medicinal use and natural occurrence, such as CAP production by *Actinomyces venezuelae* (Hanekamp and Wijnands, 2004). However, the 62nd JECFA meeting concluded that it was unlikely that CAP was produced in soils. In addition, it was considered to be unlikely that there was an environmental origin of CAP due to the persistence of CAP after its use for veterinary purposes (JECFA, 2004).
A number of recommendations were formulated at the FAO/WHO workshop mentioned above (FAO/WHO, 2004), including:

- Develop Codex MRLs based upon a list of prioritized substances (among others provided by least developed countries)
- The evaluation of drugs may take place on older data, as well as through extrapolation
- A "recommended performance level (RPL)" may be recommended with regard to the hazard
- In some cases, national MRLs can temporarily serve as Codex MRLs
- A "threshold of toxicological concern" may possibly be applied.

Historical actions taken

Table 1 provides a timeline of the regulatory measures taken by the EU regarding antibiotics in shrimp. In summary, the EU established controls on all shrimp imports from some countries after it had been observed that consignments from these countries contained either CAP or nitrofurans. Moreover, imports from China were temporarily suspended. During the same period, there were also a number of measures that pertained to, for example, poultry and other seafoods, which are not further treated here. These measures were subsequently lifted after the exporting nations had established the necessary controls and guarantees, and the EU member states' controls had been favorable.

*Table 1: Historic measures of the EU regarding antibiotics in shrimps **

Date dd-mm-yyyy	*EU decision*	*Measure*	*Background*
19-09-2001	2001/699/EC	Complete control of all imported consignments of shrimp from Vietnam and China for the presence of CAP	Measure was instigated by reports on contamination of shrimps by CAP. Decision can be revised based on guarantees and results of controls.
27-09-2001	2001/705/EC	Same as for 2001/699/EC, for shrimp from Indonesia	Same as for 2001/699/EC
30-01-2002	2002/69/EC	Suspension of imports of shrimp from China	Measure was based on shortcomings of Chinese local situation noted during an EU-inspection
27-03-2002	2002/250/EC	Complete control of all imported consignments of shrimp from Vietnam for the presence of nitrofurans	Measure was instigated by reports on contamination of shrimps by nitrofurans. Decision can be revised based on guarantees and results of controls.
27-03-2002	2002/251/EC	Same as for 2002/250/EC, for shrimp from Thailand	Same as for 2002/250/EC
02-10-2002	2002/770/EC	Revocation of controls on imports of Vietnamese shrimp	Results of controls and guarantees by Vietnamese authorities led to this revocation

Date dd-mm-yyyy	*EU decision*	*Measure*	*Background*
20-12-2002	2002/994/EC	Revocation of suspension of imports of Chinese fisheries products except for shrimps, eel, and fish from aquaculture	Suspension revoked for some products based on positive results of controls and guarantees from Chinese government. For shrimp and eel, no distinction can be made between caught and reared animals.
24-06-2003	2003/477/EC	Revocation of 2002/251 for imports from Thailand	Revocation is based on favorable results of controls and guarantees from the Thai authorities
22-07-2003	2003/546/EC	Revocation of 2001/705/EC for imports from Indonesia	Revocation is based on favorable results of controls and guarantees from the Indonesian authorities
26-08-2004	2004/621/EC	Addition of processed and peeled shrimps to list of products allowed for imports, if accompanied by Chinese certificate	China has introduced systematic and complete safety checks, among others with regard to CAP and nitrofurans

** Decisions are accessible through EUR-LEX (http://europa.eu.int/eur-lex/en/search/search_lif.html)*

What kind of actions (measurements) possible

Education and quality control

In order to provide appropriate back up and skills for local industries, it is required to set up large-scale education programs for individual shrimp farmers (*e.g.* NACA, 2002). The farmers should be educated on the effectiveness and function of antibiotics, with use of antibiotic usage manuals. In addition, the industry should be informed on the potential of using probiotics, and the possibilities of alternative cultivation methods, which require less of antibiotics due to less disease. Moreover, the establishment of a local organization of shrimp farmers with support of the government would help addressing these issues to the local farmer and hence facilitate improvement of production. Trainings can then be organized on certification of farms, in order to improve the overall quality aspects (*e.g.* Antibiotic Resistance in Asian Aquaculture Environments). Implementation of the principles of HACCP should also be stimulated, since this would resolve many challenges in registration and inside the industry. With use of HACCP-based production systems, the shrimp industry can better prevent the use of unnecessary compounds and improve controls within its system. The introduction of HACCP-based systems can be stimulated by global shrimp importers and governments. Bangladesh, for instance, implemented HACCP-principles in 1997-1998 for frozen shrimp processing.

In combination with an appropriate HACCP and water quality management program, another desirable measure is the supply of high quality brood stock. For example, Specific Pathogen Free (SPF) and Specific Pathogen Resistance (SPR) brood stocks can be bred and distributed under high-quality circumstances. These brood stocks will provide offspring that is also SPF or SPR, thereby reducing the threat of disease and facilitating its eradication, so that the use of antibiotics would no longer be required. However, the latter is only possible in well-managed farms, since otherwise the threat of disease may still come from external sources.

Regulations

Alternatively, the supply of antibiotics in Asia and other parts of the world can be regulated more stringently. At this moment, the purchase of antibiotics is generally easy and not registered. Therefore, appropriate use of the antibiotic for registered applications cannot be guaranteed based on antibiotic sales records alone. However, implementation of a system for registration would mean that at least a part of the antibiotic distribution is known. In combination with an appropriate enforcement system for the use of antibiotics, a regulatory system that controls registration and utilization of antibiotics can be developed.

Related to HACCP principles and registration of antibiotics, the governments of exporting nations could design a system which provides certification of products that are free from antibiotics used during production. This could facilitate the alignment with EU principles and regulations. However, such a certification requires more stringent enforcement of legislation and a system design for controls, and also demands instruction and training to producers.

Otherwise, knowledge on antibiotics behavior in shrimp culture is not well understood. The regulation of antibiotics could be facilitated by fundamental and applied research, in order to improve the overall knowledge. This research is necessary for currently used antibiotics as well as for newly developed antibiotics. Besides that, fundamental and applied research could facilitate a better understanding of the use of antibiotics, vaccination/immunostimulants, and traditional herbs (for immunostimulation and bacterial reduction).

In addition, there are possibilities for improvement by isolation of husbandry. In many cases, disease outbreaks cannot be controlled because the cultivation systems are connected or located closely to each other. Therefore, measures should be taken to avoid negative influences spreading from farm to farm. This may require restructuring of the industry's traditional production methods.

On a regulatory basis, the destination countries could improve harmonization of risk assessment and risk policy, especially with regard to detection limits and residues without ADI/MRL. At this moment, regulation is based on the principle of MRPLs as reliable lower thresholds for “non-zero” levels of the target compound for which a “zero tolerance” policy is pursued. However, countries with more sensitive analytical tools can have lower limits. The use of MRPLs may be too stringent for certain compounds. The abovementioned does not only apply to the shrimp industry, but could be extended to all aquaculture industries, since the problems and challenges are found in these industries, especially in developing countries.

Indicators

The problems of antibiotic use were triggered by a variety of aspects, and existed most likely before the problem was recognized as such. Many of these aspects can be extrapolated to other emerging food safety risks as well.

Lack of internationally harmonized legislation and quality assurance

One of the aspects is the absence of harmonized legislation, which results from unclearness, for example with regard to toxicity data, and a lack of synergy within the industries. Many production locations produce shrimp for multiple costumers or sell their products to whole-sellers where different lots are mixed. Due to the lack of a quality assurance (QA) system and communication, whole-sellers have difficulties in controlling the usage of compounds. In addition, shrimp farmers do not know which legislation they should comply with, and thus choose, probably without any knowledge, to use various compounds in order to improve the farm yield.

Zero tolerance and background presence of antibiotics

Other difficulties are encountered with regard to the zero tolerance level of antibiotics. Some researchers have reported that antibiotics are usually present in background levels in natural resources. This can be a result of the usage of antibiotics for human medicinal purposes, but also of production by naturally occurring bacteria in seawater and sediment. Here lies a potential and emerging problem for shrimp industries, since the improvement of detection methods will result in lower detection limits. This in turn may result in the detection of "commonly" occurring residue levels of, for example, antibiotics in shrimps. This, however, does not need to be a result of human impact, but may well be a result of natural occurrence, and thus zero tolerance is not achievable during production.

Another aspect is that there are usually no checks of unknown risk compounds, which means that even though compounds are used for several years, the problem has never been detected. In antibiotic use in aquaculture, this was not the case, since there were methods available, although the problem was highlighted when more sensitive methods were developed.

Illegal use of - or easy access to - antibiotics

At this moment there are no indications of illegal use of chloramphenicol or nitrofurans, since the Asian authorities have forbidden their use, penalties are high, and the risk of detection is also high (because authorities monitor themselves). However, the monitoring in the producing countries is very scarce in comparison to the amount of controls of importing countries. Here, all receiving countries perform import controls, and thus the risk of illegal usage being detected becomes very high. Especially, since costs for the controls, shipment etc. needs to be paid by the exporting country/industry.

Another indication of potential use of "novel" antibiotics for shrimp production is easy access to antibiotics. Many countries where aquaculture is practiced have less legislation on the availability of antibiotics. Moreover, some Asian countries are also important producers of medicinal compounds. This means that the production and distribution of these medicines is relatively cost-efficient. Therefore, the potential of using new antibiotics (even for testing purposes) may be higher in comparison to countries where antibiotics are better regulated.

Sharply increasing production and trade

One of the most important indicators is the increasing trade in - and thus production of - shrimp, as previously observed in Asia. *Figure 1* illustrates an increase in production especially from 1990 onwards. This increase in production has been realized by means of intensifying aquaculture. In general, an intensification of aquaculture will sooner or later be affected by disease pressure in the system, usually within several years. In shrimp culture, this was probably one of the most important reasons why the risk emerged. Therefore, and increase in trade and a concurrent intensification of culture systems can in this case be seen as important indicators for an emerging risk.

Many countries and local governments see market potential for aquaculture products, and therefore stimulate the local population to start producing these products. However, financial support is usually low or lacking, or developments are forced, so that the production systems are set up rapidly without regard to sustainability, best practice, or potential future problems. Therefore, problems such as disease outbreaks may be considered or detected, but improvement of the system is nevertheless too costly. The production systems may resort to treatment with chemicals or antibiotics in order to solve problems. These practices can be avoided or minimized when the production systems are evaluated and optimized before increasing the production

Since some frequently used antibiotics are prohibited nowadays, there is an increase in demand for alternatives to antibiotics. This may indicate that shrimp farmers are searching for new compounds in order to prevent disease problems that they may encounter in their shrimp ponds.

Combinations of indicators

Table 2 lists the various opportunities for synergy between indicators. It also provides information sources on the factors listed. In a number of cases, international and European databases can be drawn upon, whereas in other cases, the information needs to be obtained from source countries.

Table 2: Opportunities for synergy between indicators

Synergy	*Indicator*	*Information source*
Increase in production -> increasing disease pressure -> antibiotic use -> Risk	Increase in production	FAO EUROSTAT
Intensification of production -> increasing disease pressure -> antibiotic use -> Risk	Intensification of production	FAO
Increase in small scale production -> No education -> misuse -> Risk	Increase in small scale production No education	FAO
Increase in small scale production -> Lack of knowledge in use -> late detection -> Risk	Increase in small scale production Lack of knowledge in antibiotic use	FAO
Lack of financial means -> cheap systems -> disease pressure -> Antibiotics	Lack of financial means	LEI FAO Fisheries statistics
Rapid developments -> Quick solutions -> no preventive measures -> use of antibiotics	Rapid developments Quick solutions	Country reports
Easy accessibility chemicals -> easy use -> Risk	Easy accessibility chemicals	Production numbers Pharmaceutics
More resistant strains -> other antibiotics -> new problem	More resistant strains	Science programs
Lack of knowledge -> best fit measures -> use of antibiotics -> Risk	Lack of knowledge	Authorities source countries
No regulation -> misuse -> Risk	No regulation	Authorities source countries
Non compliant regulation -> use -> Risk	Non compliant regulation	Authorities source countries
No ADI/MRPL known -> background levels -> detection -> Risk	No ADI/MRPL known	ADI/MRPL Database
Illegal use -> few controls -> late detection -> Risk	Illegal use	Authorities source countries EFSA

Risk management

Risk management may entail the prevention or mitigation of the abovementioned problems, as has been discussed in part already under "what kind of measures possible" (see above).

For example, by harmonization of legislation on antibiotics, discrepancies between exporting and importing nations can be avoided or "repaired." In addition, issues of "zero tolerance" need to be solved, such as has been done by establishing MRPLs for compounds without ADI/MRL.

Among others, exporting nations should consider the prohibition of certain antibiotics for use in aquaculture, particularly because anecdotal evidence suggests that these compounds may be easily available for shrimp and fish farmers in third countries.

As can be learned from the incidents surrounding the EU measures on CAP and nitrofurans in shrimps from some South East Asian countries, control and certification of consignments by exporting nations before they are being shipped to overseas ports has led the EU to suspend measures. Such practice will also help preventing economic loss resulting from refusal of consignments at ports of entry.

Quality assurance of production methods may also facilitate compliance "from farm to fork" in nations where aquaculture takes place. This may entail the education of stakeholders. As stated above, there is a Dutch-Asian joint initiative underway to educate stakeholders by means of "road shows."

Important may be also for the exporting nations to realize that these activities will not only benefit exports, but also national public health. For example, the recently initiated ASIARESIST project, which is funded by the EU as a "specific support action," investigates the occurrence of resistance to CAP in cultured shrimp in South East Asia. This resistance may occur due to use of CAP in farming. It has already been observed during this project that CAP resistance occurs in isolated strains of Escherichia coli, for example, and that this resistance can be transferred to other bacteria under laboratory conditions (Chinabut et al., 2005; ASIARESIST, 2005).

In addition, a pro-active search should be carried out for antibiotics and alternatives that may be used in aquaculture for antibiotics that have been banned or to which resistance has developed. These searches should take into account a number of factors. As explained above, the sensitivity of common shrimp pathogens to an antibiotic may indicate that this antibiotic would be an attractive substitute. This should be considered in combination with other factors, such as the availability of this antibiotic to the local market, such as in the case of "generic" antibiotics, which may be relatively easily available.

At a more general level, a pro-active search for emerging risks of chemical use in aquaculture can be performed based on the qualitative indicators mentioned above. This may lead to, for example, geographical areas where problems may start emerging in the near future.

Conclusion

The case of contamination of imported shrimps with the antibiotics, *i.e.* CAP and nitrofurans, shows how the interplay between intensification of aquaculture, lack of internationally harmonized legislation, and improvement of sensitivity of detection methods may lead to the emergence of a chemical hazard. Pro-active risk management can help prevent problems with other, similar chemicals in future, taking into account the indicators and recommendations mentioned above. This may include harmonization of legislation, in particular for veterinary drugs without an ADI/MRL; controls and quality assurance in the exporting nations; education of stakeholders; and pro-active identification of potential future problems.

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Appendix 2: Case study on pesticides of natural origin

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Introduction

For many years, the most effective regime for plant pest control was the use of synthetic pesticides together with the development of resistant crop cultivars. Increased pesticide application is seen as a threat for public health and the environment, due to the relatively long environmental half-lives of halogenated hydrocarbons and the notion that their toxicity is not restricted to the target organisms. Moreover, there is a growing problem with resistance to pesticides in target pest populations. The need for ecologically safe alternatives has led to a search for naturally occurring insecticides, pesticides, and agrochemicals.

In organic farming, there is a strict regulation of the use of plant protection products, which restricts the use of the vast majority of all available compounds (Council Regulation 2092/91/EEC; this regulation and other legislative documents are available from EUR-LEX, http://europa.eu.int/eur-lex/en/search/search_lif.html). Organic agriculture in the meantime still needs some plant protection products since the availability of these products strongly affects the yield and quality of the crops. The possibility to use specific products is regulated by EU Directive 91/414/EEC, which permits countries to use some products according to national legislation.

Natural pesticides have been found in plants and minerals. Examples are pyrethrum (pyrethins), rotenone, or ryania (botanical insecticides). But also minerals can be used, such as boric acid, cryolite, or diatomaceous earth. Natural pesticides are largely insecticides.

The common assumption that "natural" means safe is not always true. Some organic pesticides are non-toxic to humans, but others are as toxic, or even more toxic than many synthetic chemical pesticides. Organic pesticides may form an emerging risk due to unexpected toxicity because of intrinsic factors or due to different behavior in the environment.

In this study, two types of organic or biological pesticides are discussed: 1) pesticides based on plant extracts and 2) pesticides based on viable micro-organisms (bio-pesticides). The latter group of pesticides is formed by the bio-pesticides, which are based on living micro-organisms used for control of disease. Moreover, the regulatory position of bio-pesticides in the EU is discussed.

The natural pesticides neem and rotenone are discussed as case studies and as examples of possible risks of natural pesticides. For neem, this is because of its apparently non-toxic behavior and long traditional use, and for rotenone because of its assumed relation with Parkinson's disease. On the basis of these studies, recommendations are formulated for the pro-active identification and prevention of emerging risks of bio-pesticides and botanical pesticides.

Classes of biological pesticides

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. In this review, only the first class is discussed in the section about bio-pesticides, whereas the third class is discussed in the section about pesticides based on plant extracts. The GMO-based pesticides fall beyond the scope of this review.

Commercially available plant-extract-based pesticides are horticultural oil, neem oil, hot pepper wax, pyrethrin, rotenone, and insecticidal soap (Higgins, 2001). Examples of bio-pesticides are based on *Bacillus thuringiensis* and milky spores (*e.g.* Bellinger, 2005).

Biopesticides

Biopesticides are an important group of pesticides that can reduce pesticide risks. In general, they have a narrow target range and a very specific mode of action, are slow-acting, have relatively critical application times, suppress rather than eliminate a pest population, have limited field persistence and a short shelf life. Moreover, they are regarded as safer to humans and the environment than conventional pesticides and as presenting no residue problems (Dewhurst, 2001).

Mechanisms of action

Biopesticides can suppress pests like insects, weeds, and plant pathogens by producing toxins specific to the pest causing a disease, preventing establishment of other microorganisms through competition, antagonism, and hyperparasitism of certain microorganisms (Montesinos, 2003).

The most common approach is to proliferate and apply high numbers of pest antagonists or pest pathogens that directly attack the target organisms. Insect viruses, weed pathogens as herbicides, and several insect- or pathogen targeted bacteria and fungi have been tested (Gerhardson, 2002). Several of such commercial products are on the market, such as a preparation of the fungus *Conithyrium minitans* for pathogen sclerotia, a strain of *Bacillus subtilis* against fungal pathogens and, most popular, strains of *Bacillus thuringiensis* for insect control (Gerhardson, 2002).

Another method is selective protection of the infection sites, which can overlap with the pest attacking approach. Here microorganisms are used that can colonize specific substrates or sites where they can compete for nutrients, space, or siderophore production with the pathogens (Raaijmakers et al., 1995).

Residues of biopesticides

When viable micro-organisms are used after application the organisms may grow or multiply and change morphologically or biochemically due to environmental influences. This is in contrast to non-viable residues, which decrease in time. This effect has to be monitored closely. Bio-pesticides' unpredictable behavior in the environment may pose an emerging risk.

Residues of plant extracts

Although natural pesticides are generally less toxic than synthetic compounds, the introduction of unnatural high levels of natural toxins could cause adverse effects. However, natural products mostly have a much shorter half-life then synthetic pesticides, which is positive for the environment, but may be a problem concerning efficacy (Duke, 1990).

Legislation on natural pesticides

In the European Union, Directive 91/414/EEC harmonizes the registration requirements of pesticides. This implies a reduction in active substances from 900 existing in 1991 to less than 400 in 2008. Registration requires the compilation of an extensive dossier, providing scientific data on toxicological studies in animals, ecotoxicology, traceability, and ecological impact (Montesinos, 2003).

This directive has been amended especially for bio-pesticides by directive 2001/36/EC.

Regulation of micro-organism-based pesticides is mentioned in the Plant Protection Products Directive 91/414/EEC. The Directive defines micro-organisms as bacteria, fungi, protozoa, viruses, and viroids, but there is no defined limit to "microscopic." Data on the micro-organisms' species, basic biology, and selection procedures are required, as well as data on assessments of risks for human health, efficacy, and environmental impact. Genetically modified organisms (GMOs) additionally fall under EC Directive 2001/18/EC, which requires risk assessment, labeling, and public information on GMOs.

EU Directive 91/414/EEC distinguishes between 3 possible stages of assessment (Dewhurst, 2001). The first stage may be sufficient, unless the information on safety is inadequate for risk assessment, in which case there is a need for additional studies concerning sensitizing potential, infectivity, genotoxicity, pathogenicity, and toxicity. If negative results have been obtained or information in stage 1 gives rise to concerns, additional investigations may be required. Since most biological pesticides have a limited applicability, these studies will be too expensive to perform.

For organic farming, Council Regulation 2092/91/EEC lists all products that can be used. On a national level, however, the products that may be used can vary quite considerably from country to country. This is because these products also have to comply with national pesticide legislation and because certain aspects of 2092/91/EEC are interpreted in different ways by different EU Member States. Many of the plant protection products allowed for organic agriculture are subject to re-evaluation under Directive 91/414/EEC. If they fail this re-evaluation, the range of allowed compounds may be reduced substantially.

Discussion

Most pesticides used to protect crops against insects are synthetic compounds. More than 300 active substances are approved for this application, whereas only few are based on biological agents. The farmers who apply these chemicals are most at risk of exposure to these agents, but also consumers may come into contact with residues on fruit and vegetables. The need for new, effective, and environmentally sound pesticides is evident.

In this study, two types of organic or biological pesticides are distinguished, products based on plant extracts and products based on viable micro-organisms (bio-pesticides).

Historically, preparations of crude natural extracts have been used as pesticides, but since regulations require a fixed chemical composition of specified compounds, these extracts containing many biological active compounds will not be suited for registration. Moreover, many natural products are so complex that their chemical synthesis will be too expensive. But in some cases, synthetic analogues may be more economic and may show similar or even superior biological activity (Duke, 1990).

Although natural pesticides are generally less toxic than synthetic compounds, unnaturally high levels of natural toxins could cause adverse effects on the environment. Natural products mostly have a much shorter half-life than synthetic pesticides, which is positive for the environment, but may be a problem concerning efficacy (Duke, 1990).

Natural pesticides are made from plant secondary metabolites and companies that exploit these plants may be tempted to optimize production of these compounds by using genetic improvement through application of classical or biotechnological methods (Duke, 1990).

Microbial bio-pesticides have a lower risk of insect resistance than chemical insecticides and their use is increasing (Sudakin, 2003). Bio-pesticides have the ability to multiply and may alter after application which may give rise to new risks. Microbial pesticides may affect the health of the consumer or people exposed to them in several ways including infectious and immunological mechanisms. These organisms are very complex and ill-understood concerning working mechanism, toxin production, specificity, and fate in the environment. This means that they may present a new type of hazard both for the environment and public health (Sudakin, 2003).

As compared to synthetic pesticides, natural plant-based pesticides are less toxic and less persistent in the environment. This advantage is at the same time a disadvantage, leading to the need for more applications, which may result in higher inputs in terms of finance and labor

General recommendations on organic pesticides

Based upon the general review and the case studies on neem tree and rotenone (see below for details), the following generalized recommendations are drawn:

Traditional, safe botanicals may form an emerging risk when new extraction methods are applied

An equal approach towards the regulatory risk assessment of natural and synthetic pesticides is needed when neurotoxicity is to be expected.

Bio-pesticides containing living organisms may present a new type of hazard both for the environment and public health due to their ability to multiply in the environment and need further investigation.

Cases on plant-based insecticides

<u>Case I: Neem derived pesticides, traditionally safe, unknown hazards of new extraction methods</u>

Introduction

The neem tree [*Azadirachta Indica* A. Juss. (Meliaceae)] grows in both Africa and India and is traditionally used to protect crops against insects. Bitter compounds are found in all parts of the tree. These compounds often show an anti-feedant effect on insects and can also affect hormonal processes in insects (Schmutterer, 1990).

Azadirachtin is the most active insecticidal compound predominantly found in the seed. Neem is a very promising insecticide (Schmutterer and Singh, 1995), which contains limnoid allomones affecting many insects. The biological effects are repellency, feeding deterrence, growth disruption, reduced viability, and disturbed reproduction (Schmutterer, 1990). Moreover, the oil blocks the tracheal openings thus suffocating the insect (Brahmachari, 2004). Neem extracts have also an antifungal activity, which can be used for fungal problems in tomatoes, rice, wheat, soy etc. (Subapriya and Nagini, 2005).

Extracts or leaves are traditionally used to protect stored seeds (maize, grain, rice, or beans) against insects. Concern has risen about whether human consumers could not be adversely affected by residues of treatment with neem products, which are widely used.

Toxicology

In animals:

Leaves and fruit kernels are applied immediately after grinding and drying and possible toxic components are not concentrated as in extracts or seed oil.

Acute effects of unprocessed material in animals are reported only for a sheep that showed nervous symptoms, dyspnoea, and hepatic failure after eating neem leaves (Ali and Salih, 1982).

In animals, there are also some data on clinical effects of neem. Leaf juice showed anti-anxiety properties in rats at low doses but not in high doses (Jaiswal et al., 1994). Kernel powder caused a decrease in the nematode egg count in sheep and an increase in body weight (Ahmed et al., 1994).

Neem leaf extract was found to be toxic in mice and guinea pigs leading to gastro-intestinal spasm, hypothermia, and death at 200-400 mg/kg (Boeke et al. 2004). Doses greater than 40 mg/ kg lead to toxicity and death in guinea pigs. High concentrations also inhibited thyroid function. Crude leaf extracts showed genotoxic effects in mice (Awashty, 2001). Chicken fed diets with 2 and 5 % neem leaf extract developed hepatonephropathy, changes in blood parameters, and reduced growth and feed conversion (Ibrahim et al., 1992). Neem bark aqueous extract appeared to be safe in rats, but lethal in snails and some fish (Osuala, 1993). Neem seed aqueous extract showed toxicity for tilapia fish (Oreochromis niloticus) and carp (Jacobson, 1995). In white leghorn chicks, mortality occurred within a day of feeding powdered neem berry extract. An aqueous extract of neem seed kernel inhibits trypsin activity in weanling rats (Jacobson, 1995).

Neem oil is the most toxic fraction, exhibiting acute toxicity in rats and rabbits with LD50 values of 14 en 24 ml/kg. Target organs are the central nervous system and lungs (Brahmachari, 2004).
Some commercial neem products such as Nimbidin, Vepacide, Margosan-O have been toxicologically tested. Nimbidin showed subacute toxicity in rats after 6 weeks of daily administration of 25, 50 and 100 mg/kg (Kanungo, 1996, cited by Brahmachari, 2004). The LD50 of intraperitoneal administered sodium nimbidate was 700 mg/kg in mice, whereas dogs tolerated up to 200 mg/kg. Large single doses led to kidney damage (Murthy and Sirsi, 1958, cited by Brahmachari, 2004). Nimbolide and nimbic acid can be lethal in rats due to dysfunction of the kidney, liver, and intestines, and a sudden drop in blood pressure (Glinsukon et al. 1986).
Azadirachtin is short-lived, easily degradable, and well-tolerated, even in doses as high as 1500 mg/kg in rats (Raizada et al., 2001). But for fish and aquatic invertebrates, it is toxic.
Vepacide, another neem based pesticide, caused changes in enzyme profiles indicating liver necrosis (Rahman et al., 2001). Margosan-O (active ingredient azadirachtin) did not induce apparent toxic effects in rats and mice (Jacobson, 1995). In aquatic environment, it had a half life of 36-48 hours when exposed to sunlight. It was not toxic to planktonic and Daphnia species, but the benthic invertebrate *Chironomus riparius* was affected (Scott and Kaushil, 1998).

In humans:
In some cases, neem oil also produced 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). Local application of 1% neem oil in children and adults did not reveal adverse effects after 1 year of exposure (Valecha et al., 1996).
For humans, the pure azadirachtin, unprocessed neem, aqueous extracts, and neem oil are the safest to use, whereas non-aqueous extracts are relatively toxic (Boeke et al., 2004). Maybe other compounds than azadirachtin are responsible for this toxicity, but the toxic components need further analysis in order to be able to draw more definite conclusions on the safety of these extracts (Boeke et al., 2004).

Residues
Neem residues may be present on stored seeds intended for consumption. Both unprocessed neem and neem seed oil are used for this application. Aqueous extracts are not recommended for this purpose as stored seeds should be kept dry, and non-aqueous extracts are expected only to leave low residues levels in the seeds (Boeke et al., 2004).
The risk of ingestion of unprocessed neem material from residues on beans by humans has been calculated by Boeke et al. (2004). If beans are not washed, the amount of powder residues would be 200 times higher than the calculated safe dose of 18.5 mg, but most powder is easily sieved off or removed by washing. Cases of toxicity in humans are not reported for crude material.
For neem oil, there are known cases of toxicity in young children, presented as toxic encephalopathy (Lai et al. 1990). Neem-oil-treated beans are expected to have residue levels of about 0.75 ml, which is higher than the estimated safe dose of 0.14 ml, but far lower than the doses that showed toxicity in children.
The pure neem compound azadirachtin is relatively non-toxic, showing a NOAEL of 15000 mg/kg in rats (Boeke et al., 2004).
In the environment, neem residues are readily degraded. Only aquatic organisms are affected by neem residues.

Conclusions

While neem is effective against insects and keeps the stored seeds in good condition, possible health effects of residues are not reported and more investigation is needed. The striking difference in toxicity between aqueous and non-aqueous extracts needs further investigation, especially because the toxic component in the non-aqueous extracts is not known. New extraction methods used for traditional safe botanical pesticides may thus give rise to new and unexpected risks.

<u>Case II: Exposure to the natural pesticide rotenone as possible contribution to the development of Parkinson's disease</u>

Introduction

Parkinson's disease is a neurological affection that impairs motor activity in patients. It is accompanied by degeneration of specific parts in the brain known as *substantia nigra* (black substance) and *striatum*. This degeneration involves, among others, the formation of cellular inclusions containing an incorrectly folded, fibrillar form of a commonly occurring neuronal protein, *i.e.* ά-synuclein, and ubiquitin. Various genetic factors are known to predispose for the development of Parkinson's disease, such as mutations in ά-synuclein or a protein that is involved with its correct folding. However, for Parkinson developed after age 50, non-genetic factors appear to be responsible, such as observed in studies with twins (reviewed by Langston, 2005).

Observations in laboratory animals

In 2000, a study was published in which rats developed symptoms and features typical of Parkinson's disease after having been exposed by intravenous infusion to rotenone, a commercial, natural pesticide (Betarbet et al., 2000). This study received attention from the media, such as New Scientist and CNN (CNN, 2000). In addition, the UK's Soil Association, an organization for organic farming, responded by posing restrictions on the use of rotenone by applicators, although it considered there was only a low risk for these workers and none for consumers (Soil Association, 2005).

Rotenone is derived from roots of plants that contain high levels of this compound, such as *Derris elliptica* and *Lonchocarpus utilis* (Soloway, 1976). It is used as an insecticide in cultivation of, for example, home gardening, and organic farming, and also for topical application on animals. Its use has been approved under Regulation 2092/91/EEC on organic farming. It is also used in some nations as piscicide, *i.e.* a chemical that is used to control fish.

The biochemical action of rotenone relates to its inhibition of NADH dehydrogenase, which is part of complex I of the mitochondrial electron transport chain. In addition, this inhibition of complex 1 might lead to additional oxidative stress, which can also be harmful to cells. Actually, before the discovery that rotenone could possibly be related to the cause of Parkinsonism, toxicity data for rotenone pertained to, for example, general toxicity and carcinogenicity (EXTOXNET, 1996).

In the abovementioned rat experiment, intravenous administration of rotenone to the animals at a dose of 2-3 mg/kg per day during a period from 1 till 5 weeks caused partial inhibition of mitochondrial complex I throughout the brains of the rats. In the *substantia nigra* and *striata* of the brains of 12 out of 25 rats, changes that typically also occur during Parkinson's were observed, such as formation of lesions, occurrence of cytoplasmic inclusions, and loss of dopamine, a neurotransmitter that is produced by these particular brain tissues. In addition, animals whose brains had been affected showed behavior typical of Parkinson's (Betarbet et al., 2000).

The reason for testing rotenone was that it was a known inhibitor of complex I, similar to another compound which was known to cause parkinsonism, *i.e.* N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

(MPTP; Friedrich, 1999). In 1982, MPTP was identified as a cause of drastic development of Parkinsonism in Californian drug addicts who had taken synthetic heroin containing MPTP as a contaminant (see review by Langston, 2005). MPTP is metabolized by monoamine oxidase (MAO) to the reactive compound 1-methyl-4-pyridinium (MPP+), which is taken up selectively by dopamine-producing brain cells where it can cause inhibition of complex I and damage leading to Parkinsonism. Contrary to MPP+, rotenone does not need to be taken up actively by brain cells given that it can pass cell membranes due to its lipophilic nature.

Indications for pesticides in general as possible cause of Parkinsonism
In addition, many epidemiological studies indicate that there is a link between previous exposure to pesticides, such as among farm workers and people living in the countryside, and the development of Parkinson's disease in later life. However, these studies cannot single out one specific pesticide that could be implicated (reviewed by Kamel and Hoppin, 2004). In fact, a pesticide like paraquat shows structural resemblance to MPP+ 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 ά-synuclein fibrils, which could give rise to the formation of typical inclusions in these cells. In addition to pesticide exposure, other environmental factors, such as exposure to microorganisms and previous infections, may be linked to the development of Parkinson's (Langston, 2005).

Implications of these results for food and feed safety
Reports on neurotoxic symptoms in rotenone-exposed mammals, such as through topical application, however, are not found in literature. This may indicate that the results of relatively high doses of rotenone administered intravenously to rats should be interpreted and extrapolated with caution. In addition, exposure of humans will primarily be through inhalation by pesticide workers or by ingestion of contaminated foods, for which intravenous administration is not a realistic model. Moreover, it should also be taken into account that rotenone is considered a relatively non-persistent pesticide, which is sensitive to heat, light and oxidation. In addition, rotenone is poorly absorbed from the stomachs and intestines of mammals, and does not accumulate in animals.
Accounts of other studies of prolonged oral exposure of experimental rodents do not indicate adverse effects on the brains of these animals. The accounts of severe human intoxications are scarce (see, for example, review of toxicity by Ling, 2003).

Conclusion
Research has shown a correlation between intravenous rotenone and Parkinson's disease in rats. This is consistent with the belief that chronic exposure to environmental toxins can increase the likelihood of the disease. The possible causal relationship between Parkinson's disease and exposure to specific chemical and biological agents is becoming increasingly clear, thanks to both increasing epidemiological evidence and improving insight into the mechanisms underlying the development of Parkinson's, especially in the last five years. Both natural and synthetic pesticides may contribute to the development of Parkinson's disease. This would call for an equal approach towards the regulatory risk assessment of natural and synthetic pesticides.
Whereas rotenone unlikely poses a realistic food and feed safety hazard per se, it may serve as a model for other pesticides that are more persistent, that can be present as residues in food and feedstuffs, and that are readily absorbed by intestines. Similar to the deliberations on the relatedness of rotenone (action)

and paraquat (structure) to the neurotoxin MPP+, it might be useful to systematically categorize which other natural products show resemblance to these compounds, both in structure and action. In addition, it should be taken into account that other pesticides may have less favorable characteristics than rotenone, which may increase the likelihood of human and animal exposure through food or feed. This would contribute to a pro-active search for other potential neurotoxins that may become emerging risks in future.

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