Food and Agriculture
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World Health
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Hazards associated with animal feed

Joint FAO/WHO expert meeting
FAO headquarters, Rome, Italy
12–15 May 2015

FAO ANIMAL PRODUCTION AND HEALTH / **REPORT 14**

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Declarations of interest

All experts and resource people were asked to complete a declaration of interest form before participation on this meeting. These were reviewed by the FAO WHO secretariat for potential conflicts of interests.

Many experts declared to be involved in national feed regulation or expressed opinions on the subject of this meeting; however, none of the interest declared was considered to present a specific conflict of interest. Two experts indicated specific interests:

Pascal Drouin, after a long career in academia where he has undertaken much research in the area of forage and related hazards Dr Drouin has recently taken up a position with a commercial entity responsible for producing silage additives. Due to his specific expertise and the importance of forage as a feed source, the secretariat was of the opinion that he could provide valuable inputs to the discussion but he did not participate in the final conclusions and recommendations related to hazards in silage;

Angela Pellegrino Missaglia has worked as a consultant for the Brazilian Animal Feed industry association, particularly in the area of feed safety and quality systems. Following the review, the secretariat was of the opinion that it did not present a particular conflict of interest and she participated fully in the meeting.

Declarations of interest of the resource people:

Paul Featherstone works for a commercial entity and is an expert in the valorization of former food stuffs into animal feed. As his direct association with the commercial sector presents a potential conflict of interest it was decided that he would participate in the meeting as a Resource Person, actively participating in the discussion and but not in the final conclusions and recommendations of the meeting.

Don O' Connor has worked in the biofuel sector for many years and more recently looking at the linkages between this sector and animal feed. As his direct association with the commercial sector presents a potential conflict of interest it was decided that he would participate in the meeting as a Resource Person, actively participating in the discussion and but not on the final conclusions and recommendations of the meeting.

The above-mentioned experts and resource persons were selected because their respective areas of technical expertise were very valuable to the meeting and of key importance to ensure a thorough discussion. Their declared interests were acknowledged by the participants. Their participation was considered necessary because the scope of the meeting included providing scientific advice and recommendations on management options to reduce risks to human health associated with animal feeding. It was decided that the interests declared by these experts should not prevent them from participating in the meeting and contributing to the discussions - including the formulation of conclusions and recommendations. Their activities were not considered to represent a potential conflict of interest in the meeting.

Abbreviations and acronyms

ANF	Anti-nutritional factor
BSE	Bovine Spongiform Encephalopathy
CAC	Codex Alimentarius Commission
DDGS	Distiller's Dried Grains with Solubles
DDT	Dichlorodiphenyltrichloroethane
DHP	1-hydroxymethyl-7-hydroxy-6,7-dihydropyrrolizine
DON	Deoxynivaleno
EFSA	European Food Safety Agency
FAO	Food and Agriculture Organization of the United Nations
GAP	Good Agricultural Practice
HACCP	Hazard Analysis and Critical Control Point
HCBs	Hexachlorobenzene
HCHs	Hexachlorocyclohexane
HSOS	Hepatic Sinusoidal Obstruction Syndrome
IARC	International Agency for Research on Cancer
LC-MS/MS	Liquid chromatography-tandem mass spectronomy
MRLs	Maximum Residue Limits
OCs	Organochlorine Pesticides
OIE	World Organisation for Animal Health
PAH	Polycyclic aromatic hydrocarbons
PCDDs	Polychlorinated dibenzo-p-dioxins
PCDF	Polychlorinated dibenzofurans

PCBs	Polychlorinated biphenyls
dl-PCBs	Dioxin-like polychlorinated biphenyls
ndl-PCBs	Non-dioxin-like polychlorinated biphenyls
PCNs	Polychlorinated Naphthalenes
POPs	Persistent Organic Pollutants
TEFs	Toxic Equivalency Factors
TEQ	Toxic Equivalency
TSE	Transmissible Spongiform Encephalopathies
WHO	World Health Organization
ZEN	Zearalenone

Glossary

Exposure assessment: The qualitative and/or quantitative evaluation of the likely intake of biological, chemical, and physical agents via food, as well as exposures from other sources if relevant (FAO, WHO, 2014a).

Feed (Feedingstuff): Any single or multiple materials, whether processed, semi-processed or raw, which is intended to be fed directly to food producing animals (FAO, WHO, 2008a).

Feed ingredient: A component part or constituent of any combination or mixture making up a feed, whether or not it has a nutritional value in the animal's diet, including feed additives. Ingredients are of plant, animal or aquatic origin, or other organic or inorganic substances (FAO, WHO, 2008a).

Feed additive: Any intentionally added ingredient not normally consumed as feed by itself, whether or not it has nutritional value, which affects the characteristics of feed or animal products (FAO, WHO, 2008a).
Micro-organisms, enzymes, acidity regulators, trace elements, vitamins and other products fall within the scope of this definition depending on the purpose of use and method of administration.

Hazard: A biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect (FAO, WHO, 2014a).

Hazard identification: The identification of biological, chemical, and physical agents capable of causing adverse health effects and which may be present in a particular food or group of foods (FAO, WHO, 2014a).

Hazard characterization: The qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with biological, chemical and physical agents which may be present in food. For chemical agents, a dose-response assessment should be performed. For biological or physical agents, a dose-response assessment should be performed if the data are obtainable (FAO, WHO, 2014a).

Pesticide: Any substance intended for preventing, destroying, attracting, repelling, or controlling any pest including unwanted species of plants or animals during the production, storage, transport, distribution and processing of food, agricultural commodities, or animal feed or which may be administered to animals for the control of ectoparasites. The term includes substances intended for use as a plant growth regulator, defoliant, desiccant, fruit thinning agent, or sprouting inhibitor and substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport. The term normally excludes fertilizers, plant and animal nutrients, food additives, and animal drugs (FAO, WHO, 2014a).

Risk: A function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard(s) in food (FAO, WHO, 2014a).

Risk analysis: A process consisting of three components: risk assessment, risk management and risk communication (FAO, WHO, 2014a).

Risk assessment: A scientifically based process consisting of the following steps: (i) hazard identification, (ii) hazard characterization, (iii) exposure assessment and (iv) risk characterization (FAO, WHO, 2014a).

Risk assessment policy: Documented guidelines on the choice of options and associated judgements for their application at appropriate decision points in the risk assessment, such that the scientific integrity of the process is maintained (FAO, WHO, 2014a).

Risk characterization: The qualitative and/or quantitative estimation, including attend- ant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterization and exposure assessment (FAO, WHO, 2014a).

Risk communication: The interactive exchange of information and opinions through- out the risk analysis process concerning risk, risk-related factors and risk perceptions, among risk assessors, risk managers, consumers, industry, the academic community and other interested parties, including the explanation of risk assessment findings and the basis of risk management decisions (FAO, WHO, 2014a).

Risk estimate: The quantitative estimation of risk resulting from risk characterization (FAO, WHO, 2014a).

Risk management: The process, distinct from risk assessment, of weighing policy alternatives, in consultation with all interested parties, considering risk assessment and other factors relevant for the health protection of consumers and for the promotion of fair trade practices, and, if needed, selecting appropriate prevention and control options (FAO, WHO, 2014a).

Risk profile: The description of the food safety problem and its context (FAO, WHO, 2014a).

Traceability/Product tracing: The ability to follow the movement of a food through specified stage(s) of production, processing and distribution (FAO, WHO, 2014a).

Undesirable substances: Contaminants and other substances which are present in and/or on feed and feed ingredients and which constitute a risk to consumers' health, including food safety-related animal health issues (FAO, WHO, 2008a).

Veterinary drug: Any substance applied or administered to any food producing animal, such as meat or milk producing animals, poultry, fish or bees, whether used for therapeutic, prophylactic or diagnostic purposes or modification of physiological functions or behavior (FAO, WHO, 2014a).

Executive summary

The expert meeting was jointly organized by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO), in line with their overall aims of securing feed and food safety and ensuring fair practices in the trade of feed and food. The objective of the meeting was to provide an updated overview of the current state of knowledge on hazards associated with feed (including feed and products of feed production technologies of increasing relevance, such as insects, former food and food processing by-products and biofuel by-products). The meeting was also intended to provide guidance on the most appropriate use of this information for risk analyses purposes; to identify knowledge gaps and to prioritize future work on the identification of potential hazards of key global concern from the perspective of human and animal health.

The need for feed for terrestrial and aquatic animals continues to rise with the increasing demand for foods of animal origin; however, the challenge is not only to meet this growing need for feed but also to ensure its safety. Feed safety incorporates the impact on human as well as animal health and welfare, which, in turn, can affect productivity. Hazards in feed may be inherent to feed ingredients as well as introduced during feed production, processing, handling, storage, transportation, and use. Hazards may also result from accidental or deliberate human intervention.

This report considers hazards in animal feed which present a risk for human health as a result of transfer from feed to foods of animal origin. It also addresses the impact of these hazards on animal health. While acknowledging the potential wider impacts of some of these hazards on animal health, welfare and productivity, and in turn on food security, the meeting did not comprehensively address these aspects but noted the need for further work in these areas. Hazards in water were considered wherever relevant in accordance with the Codex definition of animal feed. With regard to specific issues, veterinary drugs intentionally added to feed were not considered within the scope of the meeting. Antimicrobial resistance was not considered by the expert meeting as it is currently being addressed more comprehensively in other fora.

The expert meeting reviewed and discussed potential hazards in feed of chemical, biological and physical origin. While reviewing a wide range of hazards it did not prioritize any particular one or any group of hazards, because of differences in their potential presence in feed according to geographical area, production system and kind of feed (e.g. compound feed vs. pasture or forage), among others. The chemical hazards considered included persistent organic pollutants (POPs) such as polychlorinated-p-dibenzo-dioxins (PCDDs) and polychlorinated dibenzo-furans (PCDFs), dioxin-like polychlorinated biphenyls (dl-PCBs) and non-dioxin-like polychlorinated biphenyls (ndl-PCBs); veterinary drug residues; organochlorine and other pesticides; potentially toxic elements (PTEs) (e.g. arsenic, cadmium, lead, mercury); mycotoxins; and plant toxins (e.g. genotoxic pyrrolizidine alkaloids and anti-nutritionals such as glucosinolates) as well as other potential and emerging chemical hazards. The review of biological hazards considered primarily bacteria but also parasites, viruses and prions. In terms of physical hazards, radionuclides, residues of nanomaterials, micro- and nano-plastics and other relevant materials were addressed. For each of the above, the hazard as well as its occurrence in feed was described, and transfer from feed to food, relevance for food safety, impact on animal heath, and emerging issues and trends were reviewed. In addition, specific consideration was given to feed and products of feed production technologies of increasing relevance. Specific hazards and research requirements associated with the use of insects, former food and food processing by-products, biofuels (bioethanol and biodiesel) by-products, aquatic plants and marine resources as feed were highlighted. Methods of analysis, including multi-analyte methods, and sampling were also addressed and for each of the potential hazards both screening and confirmatory methods were considered.

The expert meeting recommended various measure and activities. It recommended FAO and WHO to develop guidelines for the prevention and control of hazards identified in feed to support the efforts of member countries in addressing these hazards. It recommended FAO, WHO and Member Countries and their capacity development partners to continue with and further enhance capacity development activities, especially on risk assessment and management of hazards in feed, including for feed sources and technologies of increasing relevance, to better meet domestic and international standards. Furthermore, the expert meeting recommended the Codex Alimentarius Commission to develop and update provisions addressing feed and more specifically those related to feed sources and technologies of increasing relevance to the feed sector. Certain recommendations addressed specifically feed sources and technologies of increasing relevance to the feed sector; others risk assessment of hazards in animal feed. Finally the expert meeting identified research needs and focus for future work.

Introduction

BACKGROUND

A rapidly growing population, along with an increase in urbanization and income are driving the demand for foods of animal origin. The consumption of animal products is estimated to be up to 70 percent higher in 2050 than it is currently (FAO, 2011). Concurrently, the demand for animal feed for terrestrial and aquatic animals will continue to go up. Measures to produce food and feed more efficiently and to reduce food and feed losses and wastes are necessary to face this challenge.

The challenge is not only to meet the growing demand for animal feed but also to ensure its safety.

Animal feed safety impacts on animal health, welfare and productivity, the health of feed producers, handlers and users, as well as the safety of the human food supply and the livelihood of farmers. Safe feed helps to reduce production costs, maintains or increases food quality and reduces food losses and wastes. Animal feed is an integral part of the food chain and its safety has been recognized as a shared value and a shared responsibility. Hazards in feed may be inherent to feed ingredients as well as introduced during feed production, processing, handling, storage, transportation, and use. Hazards may also result from accidental or deliberate human intervention.

Work on the application of the risk analysis framework provided by Codex Alimentarius in the field of animal feed has facilitated the further understanding of the role of animal feed safety on public health and of the importance of risk-based measures to prevent and control hazards. Hazards may be introduced through feed ingredients or via cross-contamination during production, handling, storage and transportation. The presence of a hazard may also result from accidental or deliberate (e.g. fraud) human intervention. Hazards associated with animal feed can be of a biological, chemical or physical nature and include pathogenic microorganisms, mycotoxins, heavy metals, dioxins, dibenzofurans and PCBs, residues of veterinary drugs and pesticides, and radionuclides. Previously unidentified hazards may be associated with new or increased use of certain feed or feed ingredients which are entering the production chain e.g. agro-industrial by-products (such as those of the biofuel industry), insects, food processing by-products, food wastes, etc. or through new and developing feed production technologies.

The Codex Alimentarius Commission (CAC) adopted the Code of Practice on Good Animal Feeding (CXC 54-2004) in 2004. The CAC has also adopted in 2013 the Guidelines on the Application of Risk Assessment for Feed (CXG 80-2013) and the Guidance for Governments on Prioritizing Hazards in Feed (CXG 81-2013). After completing work on these two documents, the Codex ad hoc Intergovernmental Task Force on Animal Feeding, noting the availability and ongoing emergence of new information in feed of relevance to human health, requested FAO and WHO to update the findings of the 2007 FAO/WHO Expert Meeting on Animal Feed Impact on Food Safety (FAO, WHO, 2008b). . This report aims to provide that update on hazards of relevance to animal feed and provide advice and orientation on this issue to Member Countries, to FAO and WHO and to other relevant organizations.

APPROACH

A Joint FAO/WHO Expert Meeting on Hazards Associated with Animal Feed was held at FAO headquarters in Rome from 12 to 15 May 2015. The World Organisation for Animal Health (OIE) and the Organisation for Economic Co-operation and Development (OECD) kindly joined this effort and participated as an important resource. The Expert Meeting was organized according to the principles of the FAO/WHO Framework for the Provision of Scientific Advice on Food Safety and Nutrition (FAO, WHO, 2007). A total of sixteen experts from six world regions - Africa, Asia, Europe, North America, South America and the Southwest Pacific, - were invited. The experts participated in their independent professional capacities and not as representatives of their governments, employers or institutions. The meeting elected Sabine Kruse and Dugald MacLachlan as chairpersons. The meeting was supported by a background paper prepared by Jacob de Jong and Gijs Kleter, RIKILT.

The experts considered the following information, which formed the basis for their discussion, conclusions and recommendations: (i) publicly available literature summarized in the background paper as well as additional inputs and information provided through their peers, (ii) data and information provided through a call for data, and (iii) information and expertise provided by the individual experts, and resource people present at the meeting.

OBJECTIVE OF THE EXPERT MEETING

The expert meeting was organized in line with the overall aim of securing feed and food safety and ensuring fair practices in the trade of feed and food. The specific objective of the meeting was to provide an updated overview of the current state of knowledge on hazards associated with feed (including feed and products of feed production technologies of increasing relevance, such as insects, former food and food processing by-products and biofuel by-products).

More specifically, the experts had the task to analyze scientific information and data on:

- Hazards, their sources, their levels and variability (seasonality) in feed;
- Transfer of hazards from feed to food products of animal origin;
- Emerging hazards in the animal feed chain; including hazards in feed, feed ingredients and products of feed production technologies of increasing relevance; and
- New analytical methods for the detection of hazards in feed, including rapid methods and multi-analyte methods.

The meeting was also intended to provide guidance on the most appropriate use of this information for risk analyses purposes; to identify knowledge gaps; and prioritize future work on the identification of potential hazards of key global concern from the perspective of human and animal health.

SCOPE

This report considers hazards in animal feed which present a risk for human health as a result of transfer from feed to foods of animal origin. It also considers the impact of these hazards on animal health. However, while acknowledging the potential wider impacts of these hazards on animal health, welfare and productivity, and in turn on food security, the meeting did not comprehensively address these aspects but noted the need for further work in these areas. Hazards in water were considered wherever relevant in accordance with the Codex Alimentarius definition on animal feed. With regard to specific issues, veterinary drugs or feed additives intentionally added to feed were not considered within the scope of the meeting. Antimicrobial resistance was not considered by the expert meeting as it is currently being addressed more comprehensively in other fora.

For the purpose of this report the term "feed" includes feed and feed ingredients.

Chemical hazards

Food safety hazards associated with animal feed include chemical substances such as Persistent Organic Pollutants (POPs), pesticides, and potentially toxic elements such as heavy metals.

PERSISTENT ORGANIC POLLUTANTS (POPS)

POPs are ubiquitous and lipophilic, consequently they bioaccumulate in lipid rich tissues of animals, particularly in oily fish.

Dioxins (PCDDs and PCDFs) and dioxin-like PCBs (dl-PCBs)

"Dioxins" (polychlorinated dibenzo-p-dioxins [PCDDs] and polychlorinated dibenzofurans [PCDFs]) are formed as unintentional by-products in a number of chemical processes as well as in almost every combustion process, but also exist as natural contaminants in the environment. Polychlorinated biphenyls (PCBs) are synthetic chemicals that are no longer produced but include a number of compounds, dl-PCBs, with toxicological properties similar to dioxins resulting in their consideration as a group.

The most critical effects of dioxins and dl-PCBs is on male offspring reproduction due to maternal body burden. At levels of exposure much higher than those occurring from food, these compounds may cause cancer and they were classified as carcinogens by the International Agency for Research on Cancer (IARC) but are not genotoxic.

The ubiquitous presence of dioxins and dl-PCBs in the environment from both natural and anthropogenic sources contributes to their potential presence in feed. Elevated environmental levels have been associated with soil and plant material on flood plains in industrial areas and also with soil and plant material in areas close to sources of industrial emissions. Fishmeal and fish oil produced using fish harvested from contaminated areas can also contain relatively high levels of dioxins. Industrial sources of contamination have included ball clay used as an anticaking agent in feed, lime as a neutralization agent for citrus pulp, contaminated oils, some mineral sources and most recently contaminated fatty acids. Direct drying of feed, using inappropriate fuel, is another potential source of dioxins.

In farm animals, chickens are most sensitive species showing so-called chicken oedema disease, wasting syndrome and decreased hatching of eggs. There is no specific information on adverse effects in other food-producing animals.

Addressing the food safety risks posed by dioxin and dl-PCBs in feed, requires information on the lipid content of the feed and on the congener profile of these hazards in the feed, which impacts their transfer from feed to food. In general, once absorbed, some congeners are metabolised altering the congener profile. Dioxins and dl-PCBs are only slowly eliminated and as such, levels found in edible tissues, milk and eggs are dependent on the levels in feed and also the duration of exposure. Accumulation of dioxins in liver is particularly important in the case of sheep and goats.

Non dioxin-like PCBs (ndl-PCBs)

Like dioxins and dl-PCB, ndl-PCBs are usually found in feed and food. Data on the occurrence of ndl-PCB in feed and food have been reported in different ways, for example as the sum of six PCB congeners (PCB 28, 52, 101, 138, 153, 180, often referred to as indicator PCB) or as the sum of seven (sum of six indicator PCB plus PCB 118). This inconsistency makes comparison of occurrence data challenging. The European Food Safety Agency (EFSA, 2010) found that the sum of the six indicator PCB represents about 50 percent of total ndl-PCB in food in Europe. Congener patterns in feed, particularly that of plant origin, and in edible tissue may differ considerably.

It is difficult to identify particular adverse effects in humans and animals of ndl-PCBs due to the co-occurrence of the more toxic dioxins and dl-PCBs. The adverse effects reported in laboratory animals following exposure to individual ndl-PCB are effects on the thyroid, liver and brain biochemistry, as well as immunotoxicity, oestrogenicity and reproductive and neurodevelopmental effects. Work is continuing internationally to better define the hazard associated with these compounds that are generally present at much higher levels in feed than dioxins and dl-PCBs.

Contamination of feed has occurred through the use of PCB-containing oil as a feed ingredient. As dioxins and dl-PCBs often occur together with ndl-PCBs, the sources listed under dioxins are also likely sources for ndl-PCBs. Transfer of residues from treated wood and coatings used in feed storages has been identified as a source of ndl-PCBs in feed.

Ndl-PCBs accumulate in fat, liver, fillets of oily fish and are also transferred to lipid-rich products like milk and eggs. There are differences in the uptake, metabolism, accumulation and excretion of the different ndl-PCB congeners.

Organochlorine (OCs) and other pesticides

Major representatives of the group of organochlorine pesticides (OCs) are dichlorodiphenyltrichloroethane (DDT), lindane (γ-HCH), α- and β-HCH, aldrin and dieldrin, endrin, chlordane, heptachlor, toxaphene (camphechlor), hexachlorobenzene (HCB) and endosulfan. These substances have been used extensively in the past as insecticides and are mostly present as environmental contaminants. Since 2001, the UN-Stockholm Convention on Persistent Organic Pollutants has banned or restricted the use of these OCs (Stockholm Convention, 2019). Endosulfan is one of the few organochlorine pesticides that is still in use in some countries although in 2011 endosulfan was added to the list of persistent organic pollutants to be eliminated worldwide.

The dominant toxic effects of OCs are to the nervous system and the liver. Some OCs, e.g. DDT, also affect hormonal tissues, reproduction, foetal development and the immune system. OCs can also cause liver hyperplasia and/or liver tumours in experimental animals. DDT, HCB and HCHs are classified by the IARC as possibly carcinogenic to humans (group 2B). There are relatively few data on toxicity of OCs in farm species: neurotoxicity and effects in liver have been reported in fish and ruminants exposed to HCH. Technical HCH and alpha-HCH are carcinogenic in animals whereas there is limited evidence for carcinogenicity of beta- and gamma-HCH in animals.

There is limited and declining use of OCs for crop protection in developing countries. DDT is still used in some areas to control the spread of malaria by mosquitoes. OCs are often found in feed due to their persistence in the environment. Highest levels generally occur in fats and oils of animal and vegetable origin.

OCs are generally fat soluble and transfer to fatty tissues, liver, eggs and milk. Some of the OCs bioaccumulate in animal tissues.

Feed prepared from pesticide-treated crops and deliberate addition of pesticides to control various pests, including substances containing in pesticide formulations (carriers, aggregates, additives), are beyond the scope of this report. Other sources of pesticide residues in feed include off- target movement of sprays, use of grain treated prior to seeding with fungicides or insecticides but subsequently (accidentally) utilized in feed grain, the use of feed prepared from treated crops in ways not envisaged by the regulators approving crop use (e.g. use of grains in aquaculture, use of fruit and vegetable culls etc.). In these cases, the pesticide residues could be considered a class of contaminants. Transfer, metabolism and toxicity of specific pesticides in feed to animal products is examined prior to their authorization and establishment of maximum residue limits (MRLs) for feed and animal products. However, this may not cover the extent of all plant products that may end up in feed. Additionally, if these plant products are subject to processing, residues can concentrate in by-products that may be used as feed.

NATURAL CONTAMINANTS

Mycotoxins

Mycotoxins are toxic secondary metabolites produced by fungi that readily colonize feed and food crops. Fungi are ubiquitous and formation of mycotoxins can occur throughout agricultural commodities supply chains. Contamination can occur both before and after harvest and is very dependent on environmental conditions,

Table 1: Mycotoxin contamination in feed

Toxin	Fungal genus	Disorder	Source	Occurrence
Aflatoxin	Aspergillus	Aflatoxicosis	Peanuts, maize	Australian grain-fed animals
Alternariols	Alternaria	Poor performance	Sorghum	Australian grain-fed animals
Deoxynivalenol	Fusarium	Feed refusal	Wheat	Australian grain-fed animals
Ergot alkaloids	Claviceps	Bovine hyperthermia	Ryegrass	Australian grain-fed animals
		Ergotism	Ryegrass	Grazing animals
	Neotyphodium	Fescue foot	Tall fescue	Grazing animals
Fumonisins	Fusarium	Leukoencephalomalacia	Maize	Australian grain-fed animals
Lolitrem B	Neotyphodium	Ryegrass staggers	Ryegrass	Grazing animals
Paspalanine	Claviceps	Paspalum staggers	Paspalum	Grazing animals
Phomopsins	Phomopsis	Lupinosis	Lupin stubble	Grazing animals
Sporidesmin	Pithomyces	Facial eczema	Pasture litter	Grazing animals
Unknown	Diplodia	Diplodiosis	Maize stubble	Grazing animals
Zearalenone	Fusarium	Vulvo-vaginitis	Maize, Sorghum	Australian grain-fed animals
		Infertility	Pasture	Grazing animals

Source: adapted from Bryden, W. L. 2012. Mycotoxin contamination of the feed supply chain: Implication for animal productivity and feed security. Animal Feed Science and Technology. 172:134-158.

especially temperature and water activity. Different crops, regions, and agricultural systems are at risk for contamination by a different array of mycotoxins. This is demonstrated by the fact that grain-fed animals are exposed to different mycotoxins than grazing animals (as an example see Table 1 for Australia). Moreover, some fungi produce more than one mycotoxin and some mycotoxins are produced by more than one fungal species.

Occurrence in feed

Mycotoxins are ubiquitously present in agricultural commodities, such as cereals and oil seeds. If ingested in sufficiently high concentrations, they exert severe toxic effects in humans and animals. In 2004, a global survey was launched to assess the extent of mycotoxin contamination in feed and feed raw materials. Since then, over 19 000 samples have been analysed and more than 70 000 individual analyses have been conducted. While it is difficult to infer any long-term trends on a global level, the data confirm that high mycotoxin contamination is often linked to unusual weather. Overall, 72 prcent of the samples contained detectable amounts of aflatoxins, fumonisins, deoxynivalenol, zearalenone or ochratoxin A. Co-contamination with two or more mycotoxins was detected in 38 percent of the samples. In most cases the concentrations were low enough to ensure animal health and food safety. However, co-contaminated samples with higher concentrations might still exert adverse effects due to synergistic interactions of the mycotoxins. Emerging mycotoxins and masked mycotoxins (extractable conjugates or unextracted bound mycotoxins) may also contribute to the overall toxicity of the feed and their presence is frequently detected with multi-mycotoxin liquid chromatography-tandem mass spectronomy (LC-MS/MS). Since by-product feed, such as bran, straw, distiller's dried grains with solubles (DDGS), often concentrate the mycotoxins of the original substrate, they contribute excessively to the overall contamination of feed rations and therefore need special attention.

Mycotoxins can also be found at high levels in vegetable oil by-product groundnut cake, which is commonly used as a feed component in some developing countries. New mycotoxins continue to be identified, a trend that is anticipated to increase with the use of food waste in feed.

Mycotoxins affect animal health and productivity, which on their own are significant constraints for developing countries where smallholder farmers rely on livestock for food and nutritional security; lower livestock productivity, affecting food security and nutrition, can be detrimental for human health in these areas. Some countries have differentiated levels of maximum allowable limits for mycotoxins in feed depending on age, production type and species of livestock for production of animal-sourced food, according to susceptibility of the animals and likelihood of transfer to the food products.

Aflatoxin B1 is the most potent known carcinogen, likely stunts foetal and children's physical and cognitive development, is immunosuppressive in livestock and likely in humans, and can be lethal to both in cases of acute poisoning.

Fumonisin is a threat to human health from food, but is not considered a hazard to human health from contaminated feed due to low transfer rates.

Phomopsins A, B, C, D and E are a family of mycotoxins produced by a fungus, Diaporthe toxica (formerly Phomopsis leptostromiformis). The main host for the fungus are field lupin crops. The phomopsin mycotoxins are modified polypeptides that bind with high affinity to tubulin. They disrupt microtubule function and cause a disease of livestock referred to as lupinosis.

At least one study has associated with mycotoxins in feed with reduced efficacy of vaccines, which could lead to increased animal incidence and human exposure to zoonotic diseases, and is a potentially unrecognized hazard. This warrants further investigation as it could be a significant hazard especially in developing countries where levels of multiple mycotoxins in feed can be particularly high.

Impact on animal health

Animal diseases affecting a range of systems of the body can arise from ingestion of various mycotoxins, individually or in combination, resulting in various toxicity lesions The adverse effects of mycotoxins may result in acute, overt disease or chronic, insidious conditions. Fortunately, contamination levels in food are usually not high enough to cause overt toxicoses, except in cases of episodic outbreaks in geographic hotspots. Low levels of toxins in foods are likely to result in an array of metabolic disturbances, which may or may not be accompanied by pathological changes. The effects will be unpredictable, as toxicity will depend on the toxin(s) present, dosage, duration of exposure and a variety of other factors, including animal species, age, gender, nutritional status and concurrent disease. The gut microflora may also modulate mycotoxin toxicity. At low levels, the immune system is the first to be affected, which can reduce the effectiveness of vaccines. Further, productivity is negatively affected following even low-level exposure, including growth rates, feed efficiency, reproduction/hatchability, and carcass quality.

Transfer from feed to food

The mycotoxins recognized to transfer from feed to food products at significant levels are aflatoxins and ochratoxin, with others transferring at lower levels. Aflatoxin levels in feed (and food) can be thousands of part per billion (ppb), orders of magnitude above legal limits. The biggest hazard from this is transfer into milk as a metabolized form, aflatoxin M1, as 1-7 percent of the total aflatoxins consumed by the animal; human breast milk can also be contaminated. In animals, aflatoxin M1 has been identified primarily in cow's milk. While aflatoxin M1 is less toxic than B1, the use of milk as a significant food source for infants increases the severity of this hazard. Aflatoxin is also found in dairy products such as cheese and yogurt. It has also been found in meat including fish, organ meats including liver (as B1) and in eggs. Transfer of other mycotoxins from feed to animal products have been found, typically at less than 1 percent of the consumed mycotoxin: ochratoxin A (OTA), zearalenone (ZEA), deoxynivalenon (DON), deepoxy-deoxynivalenol (DOM), fumonisins, patulins (2-3 percent), T-2- and HT-2-toxin, ergot alkyloids and citrinin. Cyclopiazonic acid (CPA), often produced in association with aflatoxin, has been shown to contaminate meat, milk and eggs.

Final remarks

There are not many longer-term studies with higher levels of mycotoxins, and multiple mycotoxins, from naturally contaminated feed, or across different breeds, age and sex, including those with other health issues; this would better reflect conditions related to feed, especially in developing countries of the tropics and sub-tropics.

The profile of mycotoxins of importance continues to evolve. Citrinin and patulin have been known for years, and with changing agricultural practices these are likely to become more prominent in the future. There are a range of known toxins that are likely to change with evolving agricultural practices, including use of different feed sources. Moreover, there are likely to be many as yet unrecognized mycotoxins, given that there are millions of fungal species, each producing hundreds of secondary metabolites.

Mitigation measures to reduce mycotoxin levels as a hazard in feed are available, with varying practicability depending on where in the world they are being considered. Good agricultural practices, plant breeding, use of less susceptible varieties, plant protection, crop rotation, and appropriate drying and storage practices can all reduce mycotoxin levels in grains. Given that these cannot realistically eliminate mycotoxins altogether, proper sampling and testing is further required to identify contaminated feed. Contaminated samples can be further subjected to visual/automated sorting (which can however exacerbate the problem, concentrating mycotoxins in feed in developing countries), decontamination (e.g. ammoniation), addition of binders (which warrant further investigation and regulation based on varying effectiveness), or the careful use of mycotoxin containing feed for animal feeding of less sensitive animal species.

Plant toxins

Plant toxins are metabolites produced by the plants. The molecular structure varies from e.g. small simple calystegines to the complex dimer protein structure of ricin. The toxins can be restricted to one family of plants but may as well be produced by several families.

In the 2007 FAO/WHO Expert Meeting on Animal Feed Impact on Food Safety, toxic plants were considered as "an undesirable substance of concern in feed". The experts defined toxic plants as plants having direct toxic effects on animal health, and the potential to transfer some toxic compounds to milk and meat. They also identified lack of information about metabolic fates, residues, maximum limits (MLs) and average daily intakes (ADI) for these different toxicants and concluded that the risk pathway can be controlled by following Good Agricultural Practices.

While some plants occur ubiquitously around the world (i.e. *Solanum* spp., *Lolium* spp.), others are restricted to certain geographical areas, such as *Indigofera* spp. that occur in tropical and subtropical regions. *Euphorbia helioscopia* or *E. nubica*, in Africa results in poisoning the dams as well as their suckling kids. Plant toxin susceptibility between animal species (considering age, size, sex and physiological stage), can differ depending on the chemical nature of the toxins, the amount and type of the toxin eaten (i.e. alkaloids, solanines, saponins, oxalates, glycosides, gossypol, etc.), parts of the plant eaten (whole, leaves, roots, seeds) the maturity stage of the plant, and the environmental and geographical area of the plant, which means that issues connected to plant toxins can be very local. Concentrations of plant toxins can vary among the season (rainy or dry) and between years, making estimations difficult. As a consequence, hundreds of plants and related toxins are reported to be relevant for animal health (Table 2).

The main routes of exposure of animals to plant toxins are through consumption by the animals while they are grazing, or through compound feed, or by feeding crop residues such as *Brassica* leaves. Toxic components can persist in hay and silage such as that containing *Colchicum autumnale, Senecio jacobaea, Equisetum* spp. *or Tiiglochin* spp.

The most important plants involved in oxalate intoxication of ruminants include halogeton (*Halogeton glomeratus*), soursob (*Oxalis* spp.), rhubarb (*Rheum rhaponticum*), curly dock (*Rumex crispus*), purslane (*Portulaca oleracea*), lamb's quarter (*Chenopodium album*), bassia (*Bassia hyssopifolia*), greasewood (*Sarcobatus vermiculatous*), pigweed (*Amaranthus* spp.), Russian thistle (*Salsola kalis*) and sugar beets (*Beta vulgaris*). Species of grasses in the genus of *Cenchrus*, *Panicum*, and *Etaria* which are widely cultivated in tropical and subtropical areas can also accumulate toxic amounts of oxalate. Other plants causing liver disease and photosensitation (sensitivity to sunlight) are often grouped together, as photosensitivity is often a secondary symptom of liver disease caused by poisonous plants (*Allium* spp., *Hypericum perferatum*), other plants contain pyrrolizidine alkaloids, causing muscle degeneration, liver necrosis, death (*Thermopsis rhombifolia, Amsinkia intermedia, Senecio* spp., *Symphytum* spp.) or contain (*Conium maculatum*) neurotoxins as piperidine alkaloids. Other plants may contain cyanogenic glycosides compounds that are converted to hydrogen cyanide or prussic acid causing neuronal disorders, lack of coordination and death (e.g. *Acroptilon repens, Centaurea solstitalis*).

Of particular importance are pyrrolizidine alkaloids. 1,2-DehydroPAs are not directly toxic but are metabolized by cytochrome P450 enzymes in the liver to 1-hydroxymethyl-7-hydroxy-6,7-dihydropyrrolizine (DHP) ester metabolites (DHP esters). DHP esters are powerful bi-functional alkylating agent and they rapidly form DHP adducts with, and cross-link DNA and proteins, in the liver. They cause somatic mutations and liver cancers. The DHP esters formed in the liver are also hydrolyzed to DHP which is a less reactive bi-functional alkylating agent than its precursor esters. DHP escapes from the liver, circulates and alkylates DNA and other nucleophiles in many tissues. When DHP is injected subcutaneously into rats, rhabdomyosarcoma is produced at the site of injection in 65 percent (39 of 60) of the animals. Cancers of the lung, kidney, skin, intestines, bladder, brain and spinal cord, pancreas, adrenal gland and leukemia have also been produced by 1,2-dehydroPAs and their metabolites in experimental animals and DHP adducts have been detected in all of these tissues. DHP is considered to be possibly responsible for all of the cancers occurring beyond the liver in animals exposed to dietary 1,2-dehydroPAs.

The alkylating potential of DHP esters and DHP does not however end with the formation of the initial DHP adducts. Some DHP adducts are reversible under physiological conditions and it is believed that these and circulating mono-DHP adducts, form a reservoir of ongoing alkylation potential *in vivo*.

As well as cancers, certain 1,2-dehydroPAs, such as monocrotaline, are very commonly used to produce animal models of progressive pulmonary arterial hypertension leading to right heart failure. 1,2-DehydroPAs also cause hepatic sinusoidal obstruction syndrome (HSOS) leading to cirrhosis.

Occurrence in grasslands/pastures and rangelands

Pastures often contain weeds that are potentially dangerous to livestock. The toxic compounds in plants are usually a defence mechanism against predation and have a distinct, unpleasant odour or a bitter taste and are not preferentially grazed. Consumption of unpalatable plants will increase under some circumstances, primarily if other forage is not available. Some plants, like those that accumulate nitrates (i.e. *Sorghum bicolour, Sorghum halapense, Chenopodium* spp., *Amaranthus* spp.), can increase in toxicity after rainfall or on cool, cloudy mornings and evenings.

Animals for dairy production are kept close to the milking parlour and often graze on cultivated grassland. Their diet is, in general, amended with preserved roughage (hay or silage) and compound feed (concentrates) which in some cases the toxic plant has been conserved in association with the native grasses (e.g. *Colchicum autumnale*). Grazing management is a critical component to maintaining pastures free of poisonous weeds.)

Animals for meat production are raised on extensive natural pastures in many parts of the world. These animals are exposed to the regional flora and native poisonous weeds (e.g. S*enecio* spp., *Cynoglossum officinale*).

Poisonous plants that impair normal reproductive functions, interfere with most reproduction processes in livestock. *Lupinus* spp., *Pinus ponderosa, Veratrum californicum, Astragalus* spp. and *Oxytropis* spp. contain indolizidine alkaloid, *Conicum maculatum, Lupinus* spp. and *Nicotiana* spp. contain quinolizidine and piperidine alkaloids that are fetotoxic when grazed by pregnant cattle during specific stage of gestation. Mimosa tenuiflora can cause embryonic death.

Occurrence in compound feed

Soy and other leguminous such as lupine, are commonly used in compound feed for farm animals and fish worldwide. These feed materials have a history of safe use often after treatment to reduce the anti-nutritional factors (ANF). Despite regulatory limits in the European Union, several regulated plants were detected in compound feed. Residue materials of oil production, such as oilseed meals, usually contain ANF and must be processed before use. The variability in concentrations make some varieties of flaxseed more suitable for use as broiler feed than other varieties due to differences in ANF (cyanogenic glycosides, phytic acid, condensed tannins and trypsin inhibitors).

Herbs

Herbs are sometimes added to feed for pharmaceutical purposes, as flavours in feed as well as to confer specific flavours to the meat. Herbs are currently not identified as hazards associated with their use in feed. Given that the use of herbs are used extensively by the feed industry and may continue to rise in the future, potential hazards should be assessed as appropriate.

Effects of processing

Processes for raw materials to be used in compound feed often are heat and fermentation to reduce the ANF or phytoestrogen activity. Sometimes organic solvents are used to reduce ANF.

Silage process may reduce the amount of toxins, e.g. pyrrolizidine alkaloids or tannins from sorghum. Sun drying processing techniques reduce only 60 to 70 percent of the total cyanide content in cassava (FAO, 1990), the reduction of cyanides depends on whether the product is first placed in cold water (27°C) or directly into boiling water (100°C).

Some data on occurring plant toxins in wild plants are reported for e.g. swainsonine and calystegine in *Ipomoea carnea*, ANF in flax seed flours, and the saponin protodioscin in several species of *Bracchiaria*, *Panicum* and *Andropogon*. Many lists of poisonous plants in various regions of the world has been cited e.g. for Saudi Arabia, Australia, United States of America, Netherlands, Brazil and Europe (Table 2).

Transfer to food of animal origin

Milk is a product excreted by the mammary gland, and also is a route of excretion of toxic components e.g. pyrrolizidine alkaloids, glucosinolates. The ingestion of *Senecio* spp*., Crotalaria* spp*., Heliotropium, Echium,* white snake (*Eupatorium rugosumn*), rayless goldenrod (*Haplopappus heterophyllus*), *Colchicum autumnale* has been detected through toxins in dairy milk. Poisoning by *Ipomoea asarifolia* in lambs by the ingestion of milk from ewes that ingest the plant has been reported. Transfer of plant toxins to meat can occur, e.g. indospicine (an amino acid, analogue of arginine, occurring in the free form only) and to eggs e.g. usaramine (a pyrrolizidine alkaloid). Parthenium hysterophorus can taint meat (unknown cause), thus reducing the value of the products.

1,2-Dehydropyrrolizidine alkaloid in feed may transfer to animal products. Hundreds of 1,2-Dehydropyrrolizidine alkaloids (1,2-dehydroPAs) are produced by many thousands of plant species that commonly grow in agricultural production systems throughout the world. They are found in rangeland and pastures and they can also contaminate grain and grain-based livestock feed.

Effects on animal health

All farm animals and fish (salmon, trout) can be affected by plant toxins. The effects of the toxins on animal health are likewise complex, varying from acute toxicity, with several more recent examples presented in Table 2, to genotoxic carcinogicity (pyrrolizidine alkaloids) to more general described effects as anti-nutritional factors caused by presence of compounds such as glucosinolates, saponins, tannins alkaloids, including pyrrolizidine alkaloids, and terpenes, tannins and cyanogenic glycosides. Overall effects from exposure to plant toxins include: reduced growth, egg production and milk yield, reproductive effects and immunomodulation causing increased vulnerability to contagious diseases.

Final remarks

Toxin-producing plants may occur in grasslands used for forage. Naturally occurring toxins can include pyrrolizidine alkaloids (e.g. jacoline from Senecio jacobaea) and other alkaloids (e.g. atropine, cocaine, ephedrine, morphine, nicotine, solanin), terpenes (e.g. camphor, pinene), tetrahydrocannabinol, gossypol, isoflavones, and glycosides (e.g. cyanogenic glycosides, digitalis), glucosolinates, ricin, theobromine in feed, tropane alkaloids in feed and saponins in feed. Transfer of some of these toxins to edible products such as milk and meat has been demonstrated.

The impact of plant toxins continues to evolve with changes occurring plants and concentrations of plant toxins due to climate change. Increased salinity of soils, due to prolonged periods of draught or expansion of cultivation land, can lead to accumulation of oxalate and coumarin in plants. Furthermore, yearly variations in climate will influence the abundance of certain plants in a region and thus increase risks in certain periods. Worldwide an increased occurrence of several weeds such as locoweed and Parthenium hysterophorus has been observed which results in a spread of the accompanying risks.

Table 2: Examples of intoxications of farm animals due to ingestion of plant toxins

Animal	Symptoms	Plant related	Toxin related	Country	Reference
Farm animals	Acute renal failure, gastrointestinal signs and cardiac dysrhythmias	Oleander *(Nerium oleander)*	Oleandrin and oleandrigenin	USA	Kozikowski *et al.* 2009 Forero *et al.* 2011
Cattle, sheep, horses	Feed intake reductions, loss of weight and fertility	Locoweed *(Astragalus* spp. *and Oxytropis* spp.*)*	Swainsonine (trihydroxy indolizidine alkaloid)	China	Chenchen *et al.* 2014
Farm animals	Neurological disease	Ipomoea carnea *(fungal endophyte)*	Swainsonine and calystegines	Brazil	Cook *et al.* 2015
Cattle	Cattle collapse, can be fatal	*Anderson Larkspur Delphinium andersonii*	Diterpenoid alkaloids	USA	Pfister *et al.* 2011
Cattle	Crooked calf syndrome; fetal movement stops, causing the foetus to grow in contorted positions	*Velvet Lupin – Lupinus leucophyllus Douglas ex Lindl*	Anagyrine alkaloid (Quinolizidine)	USA	Ralphs *et al.* 2011
Sheep	Acute respiratory distress	*Galega officinalis*	Unknown	Belgium	Dierengezondheidszorg_ Vlaanderen 2013
Camel, goat, sheep, cattle	Bloating, diarrhoea, stunted growth	*Pavetta gardeniifolia*	Not described	Africa	Adebe *et al.* 2012
Farm animals	Bloating, violent tremors, death	*Sorghum bicolour* *Sorghum halapense* *Chenopodium* spp. *Amaranthus* spp *Malva neglecta*	Nitrate/Nitrite	World	Adebe *et al.* 2012
Farm animals	Blindness, convulsions, death	*Solanum* spp.	Solanine	World	Adebe *et al.* 2012
Goat, sheep, cattle	Death	*Acokanthera schimperi*	Cardiotoxic glycoside ouabain	Africa	Schelzer and Gurib Fakim, 2008
Farm animals	Death	*Amsinckia* spp. *Senecio japobaea* *Senecio* spp.	Pyrrolizidine alkaloids	North America	Forero *et al.* 2011
Farm animals	Nervousness, muscle weakness, paralysis, death	*Delphinium* spp.	Diterpenoid alkaloids	North America	USDA, 2015 Cook *et al.*, 2015
		Oxalis spp. *Rumex* spp. *Amaranthus* spp. *Rheum* spp.		South America	

Animal	Symptoms	Plant related	Toxin related	Country	Reference
Goat, sheep, cattle	Death	*Triglochin* spp.		North America	Forero *et al.*, 2011
Farm animals	Coordination disorders, death	*Prunus virginiana* *Prunus serotine* *Acroptilon repens* *Centaurea solstitaliss* *Triglochin* spp.	Cyanogenic glycosides	North America	Forero *et al.*, 2011
Goat, sheep, cattle	Death	*Lolium* spp.	Alkaloids nitrates	World	Forero *et al.*, 2011
Sheep	Death, azotemia, hypocalcaemia	*Cicuta douglassi*	Cicutoxin (a neurotoxin)	North America	Reza Aslani *et al.*, 2011
Cattle	Teratogenic effects, reproductive effects	*Lupine* spp.	Piperidine alkaloid, quinolizidine	North America	Lee *et al.*, 2006
Farm animals	Vertigo, vomiting and collapse	*Cassava*	Linamarin (cyanogenic glycosides)	World	FAO, 1990
Farm animals	Death	*Colchicum autumnale*	Alkaloids	Europe	Winter *et al.*, 2011
Cattle	Neurologic disease	*Ipomoea carnea*	Swainsonine	South America	Cook *et al.*, 2015; Lu *et al.*, 2015
Sheep	Neurologic disease	*Ipomoea asarifolia*	Swainsonine	World	Kleber *et al.*, 2014
Farm animals	Anorexia, ruminal indigestion, oedema in lips, tongue and face	*Centhatherum brachylepis*		South America	Medeiros *et al.*, 2009
Horses	Anorexia, sleepiness, ataxia, weakness, stumbling	*Indigofera lepesdezoides*	Indospicine	North America	Lima *et al.*, 2012
Goat, sheep, cattle, pigs	Nervous signs, hypersensitivity, restlessness, stumbling gait, tremors, recumbence, tetanic and clonic convulsions, opisthotonos, teeth grinding, dyspnoea, salivation, vomiting, death	*Marsdenia hilariana* *M. megalantha*	Glycosides and alkaloids	South America	Pessoa *et al.*, 2011

* Farm animals are related with camel, goat, sheep, cattle and horse

VETERINARY DRUG RESIDUES

The issue of veterinary drug residues in feed and food has long been recognized due to long standing concerns for public, animal and environmental health as a result of direct exposure to these residues and concerns that residues of antimicrobials may be associated with the development of antimicrobial resistance.

The authorized use of veterinary drugs in feed is outside the scope of this report. However, during manufacture the unintentional cross-contamination of veterinary drugs to subsequent feed can occur. Additionally, feed produced from crops fertilized with bio wastes such as manure from treated animals may result in take up of drugs by plants and subsequent incorporation into feed. Other sources of veterinary drug residues in feed include low levels of antimicrobials in fermentation products such as DDGS. Another source of low levels of veterinary drugs in feed is their natural occurrence as some antibiotics are produced by organisms present in the environment. In these cases, the veterinary drug residues could be considered a class of contaminants.

Feed remains a much-used vehicle for the efficient delivery of veterinary drugs to animals. While transfer, metabolism and toxicity of veterinary drugs in feed to animal products is fully assessed as part of the authorization process and establishment of MRLs, the expert meeting noted that this does not cover the different non-target species which may be exposed via cross-contamination of feed, and this may be an important consideration for risk management in some countries.

POTENTIALLY TOXIC ELEMENTS (PTES)

Arsenic

Inorganic arsenic compounds are highly toxic; whereas organic arsenic compounds are much less so. Toxicity also depends on the valency of arsenic; trivalent arsenic is more toxic than pentavalent arsenic. Inorganic arsenic is classified by the IARC as a human carcinogen.

Arsenic levels vary greatly in potential feeds, but are generally high in marine organisms including fish. There are many chemical species of arsenic in fish, and the dominant form in fish is arsenobetaine, which is considered non-toxic to humans and is excreted rapidly, unmetabolized. Feed concentrations of arsenic in fish based ingredients reflect the amount and source of fish meal included. Groundwater aquifers in some areas of America and Asia have naturally high levels of arsenic. These chemicals enter the food and feed chain, and are present in water and air. Arsenic levels in plant derived materials depend on soil levels and characteristics, arsenic compound(s) present, plant species and arsenic levels in water used for irrigation. Exposure of animals to inorganic arsenic via drinking water is much higher than via feed in those areas where naturally polluted ground water sources are used.

Signs of acute intoxications of mammals with inorganic arsenic include diarrhoea, vomiting, salivation and abdominal pain. In poultry, a decrease in feed consumption and neurological symptoms prevail. In fish, the main target organ of inorganic arsenic is the liver.

Transfer of inorganic arsenic from feed to animal products (mammals, poultry, and fish) is low. In mammals, inorganic arsenic is metabolized into organic arsenic. The contribution of terrestrial animal products to human exposure to arsenic is considered insignificant.

Cadmium

Cadmium causes adverse effects in kidneys, skeleton and the respiratory system in humans and animals.

Levels of cadmium in plant-based feed depend on levels in soil, soil characteristics, use of phosphatic fertilisers and plant species. Mineral supplements, such as zinc oxide, have occasionally be shown to contain unacceptable cadmium levels.

Transfer of cadmium to muscle of livestock, including fish, is generally low, whereas significant levels can occur in crustaceans. Cadmium present in feed materials and ingested soil accumulates in kidney and liver of livestock. As the elimination of half-time of cadmium in livestock is very long, the duration and level of exposure determine levels in these organs.

Mercury

Organic mercury, mainly methylmercury, is far more toxic than inorganic mercury. The critical effect of inorganic mercury is renal damage; organic mercury main adverse effects are on the nervous system. Methylmercury is biomagnified up the marine food chain, and highest concentrations are found in predatory, large fish.

Fishmeal is an important contributor of methylmercury in feed for some terrestrial and aquaculture species. Bait fish may be a significant source of methylmercury for certain marine culture predatory fish such as tuna. Mercury levels in plant-based feed are very low.

Levels of methylmercury in terrestrial farm animals are usually at or below the limit of quantification (LOQ). In aquaculture salmonids, fish meal is the dominant source of methylmercury.

Lead

Lead effects neurodevelopment and acts on the nervous system and gastrointestinal tract. The major source of lead exposure to grazing animals is ingestion of soil. Mineral supplements such as zinc oxide, have occasionally been shown to contain unacceptable lead levels. Neurobehavioral signs are a first indicator of lead poisoning in calves. Administration of lead acetate via drinking water had adverse effects on feed intake and body weight gain of broilers. Transfer of lead to muscle of livestock, including fish, is generally low. In terrestrial species lead accumulates in bones, kidney and liver, whereas transfer to milk is low.

Other potentially toxic elements

Other elements such as selenium, chromium, copper and nickel may be of concern depending on the speciation and level of contamination in feed.

OTHER POTENTIAL AND EMERGING CHEMICAL HAZARDS

A range of contaminants including brominated flame retardants and perflourinated compounds, have been shown to be present at low levels in animal feed however there is insufficient information regarding their toxicity and transfer to edible tissue or milk, to assess whether these contaminants are hazards to consumers or farm animals.

Biological hazards

There is a continuous risk for contamination of feed by microbial pathogens throughout the production chain up to feeding to the animals. Such diverse sources for contamination make it difficult to control or fully eliminate specific pathogens. The ultimate objective of recommended risk mitigation options is therefore to produce a feed where the microbial contamination is kept at such a level to ensure that animals fed do not become infected with risk for a subsequent spread within a herd and to the environment and ultimately to consumers.

Hazard Analysis and Critical Control Point (HACCP) programs and Good Agricultural Practices (GAP) are utilized to control pathogenic agents in feed and requires insight in the origin and production procedures applied for different feed ingredients, in feed mills and at the farm level and also of complex structure of their interactions. Nevertheless, in order to identify and characterize pathogens, understanding the factors that affect the sources and routes of contamination remains crucial in order to prevent further contamination.

As an example, *Campylobacter jujeni* is a frequent intestinal contaminant of broiler chickens and, less frequently, of other farm animals. This bacterium is in many countries the most frequent source of human foodborne infection. In the case of *Campylobacter* contamination of chickens, feed ingredients are currently not a major concern when evaluating contamination route, as their characteristics often do not allow for growth of this pathogen. However, changes in rearing and feeding practices and use of antimicrobials may influence the ecological conditions for survival and growth of the microbe.

Manure use on crops grown for feed may result in contamination of feed and feed ingredients by several pathogens. Managing environmental diffusion will involve better knowledge of survival of those organisms under environmental stresses (temperature, irradiation, desiccation). Changes in rainfall or maxima and minima of temperature will contribute to influence survival. Even considered those changes, it will always be safer to increase time between application and harvesting of feed.

Increases in warmed temperature in relation with climatic changes will required more frequent utilization of animal heat stresses reduction techniques, especially for indoor maintained herds. Those techniques could induce diffusion of aerosols containing pathogens, increasing contamination to pathogen free animals.

SALMONELLA SPP.

Salmonella is a bacterium and more than 2500 serotypes of the microbe are reported. All serovars are considered as potential human pathogens. *Salmonella* is recognized as one of the most important causes of foodborne illnesses in humans. *Salmonella* is an important pathogen also in animals. In both humans and animals, the infection is transmitted by the faecal oral route.

Occurrence in feed

Wide spectra of serovars of *Salmonella* can be isolated from feed, including those most commonly isolated from clinical cases of human salmonellosis, like subspecies typhimurium and enteritidis.

Impact on animal health

Animals acquire infection following ingestion of faecally contaminated feed. Infected animals often become silent carriers without clinical sings of disease, but in particular some serovars can cause severe disease and economic losses. Infected animals shed the microbe and constitute a potential source for the spread of the infection to other animals, including wildlife, and of the environment. The importance of feed as the source of the infection is well established and in the EU a recent quantitative risk assessment concluded that infected incoming pigs and *Salmonella*-contaminated feed are the two major sources of *Salmonella*. A similar situation also applies for poultry.

Relevance for food safety

Human salmonellosis is most often contracted from food of animal origin with meat, poultry, eggs and milk are common sources. Seafood from contaminated water and unwashed fruits and vegetables can also be sources. The faecal contamination of meat occurs at slaughter and on-farm for eggs and milk. Faecal contamination of water used for irrigation can be a source of contamination of vegetables and crops. Contamination of animal feed to act as a source of *Salmonella* infections in humans was demonstrated when *Salmonella* Agona contaminated fishmeal used as feed material was estimated to have caused 1 million human cases in the United States alone up to 2001.

Final remarks

Data from targeted monitoring of the occurrence of *Salmonella* contamination of feed should be taken with caution because they often are based on non-harmonized sampling and testing procedures. Due to uneven distribution of *Salmonella* contamination in feed materials, the sample prevalence may underestimate the true batch prevalence and a low sample frequency should not necessarily be taken as evidence for low prevalence at batch-level. This is an important gap of knowledge for risk assessment of the important of *Salmonella* contaminated feeds in individual countries. There is also a need for guidelines for the sampling of feed for bacterial contamination, in particular for *Salmonella*.

The major risks for *Salmonella* contamination of feed mills and animal feed are the introduction of *Salmonella*-contaminated feed ingredients. In all countries, there is a continuous risk for introducing *Salmonella* to the food chain. The prevention and control of feed mills should be HACCP based and include continuous monitoring and actions taken when *Salmonella* contamination occurs. Control measures include prevention of contamination, reduction of multiplication and procedures to kill the pathogen. A major focus should be to prevent the introduction of contaminated feed ingredients with the major risk feed ingredients being animal derived protein followed by the vegetable proteins, the latter including e.g. soybean meal which are produced in crushing plants. A HACCP-based approach for the control can also be used in crushing plants.

A wider implementation of these measures would substantially minimise the risk for animal and human health associated with *salmonella* contaminated feed.

In the future, the introduction to the food chain of new virulent strains like *Salmonella* Agona may occur as well as a shift in the virulence of a certain serovar as occurred during the late 1980s with *Salmonella* enterididis. Those events resulted in pandemics with several of millions human cases of salmonellosis and those serovars are since established as some of the most common cause of human salmonellosis. In a similar way *Salmonella* strains with a severe panorama of antibiotic resistance may occur. Measures are therefore urgently needed to be implemented also to prevent the introduction to the feed and food chain of new emerging virulent strains of *Salmonella*.

LISTERIA MONOCYTOGENES

Listeria monocytogenes, a Gram positive bacillus, is ubiquitous and human exposure is thought to be frequent, but the incidence of infection is low and dependent on individual susceptibility; for example, the young, old, pregnant, and immunocompromised persons are found to be more susceptible. Listeriosis is linked to infection of white blood cells, leading to meningitides, septicaemia or abortion.

Occurrence in feed

Some sources of contamination from *L. monocytogenes* include soil, sewage, forage, and water. With respect to unprocessed feed, such as plant materials that either are not or are minimally processed, such as silage, *L. monocytogenes* can have the opportunity to grow out if production is not properly controlled. Brewer grain and other processed plant material could also harbour significant listeria cells.

Impact on animal health

L. monocytogenes in silage is noted as related to animal listeriosis and asymptomatic carriage in dairy cattle, sheep and goats. *L. monocytogenes* has been detected in poultry feed both prior to and after heat treatments. The feed mill has been suggested as a source of *L. monocytogenes* indicating potential re-contamination of pelleted feed. The prevalence of *L. monocytogenes* in animal feed is believed to be low in ingredients with a low water activity such as hay and cereal grains.

Relevance for food safety

Contamination of human food is mainly related to processed meat, including deli meat, as well as milk and cheese. The ability of this organism to tolerate and grow under refrigeration temperature range lower controls by food preservation methods.

ENTEROHEMORRHAGIC *ESCHERICHIA COLI*

Escherichia coli (*E. coli*) is a Gram negative bacterium that is commonly found in the intestinal flora humans and warm-blooded animals. Enterohaemorrhagic *E. coli* (EHEC) can cause severe foodborne disease.

Occurrence in feed

E. coli O157:H7 has rarely been detected in cattle feed, however some literature suggests that feed may be a source of *E. coli* O157:H7 in cattle. Time/temperature combinations that were applied in commercial pelleting processing had been investigated and it was concluded that these would not effectively kill *E. coli* O157:H7 if high numbers were present in feed.

Impact on animal health

The main reservoir appears to be ruminants, with cattle being the principal. Other animals (pigs, horses, and chicken) have occasionally been infected without any clinical signs of disease.

Relevance for food safety

Foods of animal origin, meat products, raw milk or soft cheeses made from raw milk are recognized as foods that are considered to carry a high risk of infection. These food sources are concurrent with previous outbreaks and such outbreaks were attributed to fundamental errors in food hygiene concerning heat processing and adequate cooking. The organism produces toxins, known as verotoxins that produce abdominal cramps and diarrhoea.

Final remarks

Experts acknowledged that pre-harvest controls can help to reduce, and thus manage *E. coli* contamination, however complete elimination based on such controls is unlikely and accordingly procedures during processing are still required.

CLOSTRIDIUM PERFRINGENS AND *CLOSTRIDIUM BOTULINUM*

Clostridium perfingens is classified into 5 types (A-F). Type B-F are worldwide causing serious losses in ruminants when exposed to contaminated feed on pasture if not protected by vaccination. Here we consider type A reported as one of the most common food poisoning agents worldwide. *Clostridium botulinum* produced the most potent toxin affecting animals. Both are anaerobes, spore-forming Gram positive bacillus.

Occurrence in feed

C. perfringens type A has been commonly reported in faeces and soil. They could be present as either vegetative cells or endospores in soil-contaminated feed. *C. perfringens* is commonly isolated from the environment and the intestinal tracts of animals. However if present as a contaminant in feed it may cause tissue necrosis, bacteraemia and gas gangrene.

Clostridium botulinum can be found in the soil and grows well at micro-aerobic conditions. *C. botulinum* intoxications have been reported in cattle and equine with common sources originating from the toxins produced by the bacteria in silage. A well-recognized source of outbreaks is when animals, rodents, birds or cats for example, are accidently introduced in the silage at harvesting. Contamination of pastures by poultry litter or other contaminated manure contributes to increase soil population of this organism.

Impact on animal health

Clostridium perfingens Type A can cause occasionally serious and fatal diseases in different animal species and causes sever necrotizing enteritis in chickens. The cause of botulism in animals is connected to the presence and multiplication of the *C. botulinum* toxin present in animal feed. There are 7 types of *C. botulinum* toxins (Types A, B, C1, D, E, F and G). Botulism is a rapidly fatal motor paralysis caused by ingestion of toxin.

Relevance for food safety

The bacteria are found in the environment as well as the intestines of human and animals. *C. perfingens* can be found on raw meat and poultry. Food poisonings due to *C. perfingens* can arise in food of animal origin when meat or poultry is poorly prepared (e.g. undercooked) or spores survive the cooking processes. Also, if cooked food is improperly stored (e.g. prolonged storage at room temperature), spores can germinate and rapidly multiply. Different toxins tend to be responsible for the disease in animals and humans.

Final remarks

Botulism cases in humans are generally caused by toxin types A, B, E and rarely F and are usually not linked to animal production.

BRUCELLA SPP.

Brucellosis is a zoonotic disease caused by pathogens of the genus *Brucella*, a Gram negative small coccobacillus. Human can also contract brucellosis if they come in close contact with infected animals such as sheep, cattle, goats, pigs, or dogs (e.g. an occupational disease of farmers, herdsmen, veterinarians, and slaughterhouse workers) or through animal products like milk, milk products, or undercooked meat that are contaminated with *Brucella* spp.

Occurrence in feed

Brucellosis is very widespread in many regions of the world. *Brucella* may spread in several ways including through direct contact with infected tissues or fluids of infected animals, consumption of colostrum or milk from infected animals, or consumption of feed or water that has been contaminated from infected tissues or fluids.

Impact on animal health

Bovine brucellosis, caused by the bacterium *Brucella* abortus, is an economically important cause of abortions in cattle. *B. abortus* also affects other species including bison, buffalo and elk; some species are maintenance hosts for this organism. Other *Brucella* species include *Brucella* melitensis and *Brucella* suis, responsible for chronic inflammatory lesions in the reproductive organs of ovine and swine.

Relevance for food safety

In humans, brucellosis can be a serious, debilitating and sometimes chronic disease that may affect a variety of organs. Most cases are the result of occupational exposure to infected animals, but infections can also occur from ingesting contaminated dairy products. In addition, *B. abortus* could be used in a bioterrorist attack.

MYCOBACTERIUM

Mycobacterium species, mixed-Gram bacillus from the *Actinobacterium phylum*, are responsible for several important human and animal chronic diseases worldwide, as pulmonary, skin, and intestinal tract colonizer. Some recognized diseases in human are tuberculosis, leprosy, or ulcerative colitis. The *Mycobacterium* avium complex strains are associated with tuberculosis and very common in food, water and soil.

Occurrence in feed

Mycobacterium are widespread in the environment, particularly in aquatic reservoirs. Sources of contamination of feed are numerous and include animal carcasses accidentally harvested simultaneously with fodder, and soil particles. *Mycobacterium* easily survives in more acidic soil, in a state of vegetative dormancy. This organism does not survive drying processes, i.e. in hay or grain, but it was reported that *Mycobacterium* avium subspecies paratuberculosis is able to survive in grass silage. Spreading of contaminated manure from infected farms could be a source of this bacterium. Application of manure prior to emergence of crops represents a low risk practice.

Impact on animal health

Mycobacterium bovis, *Mycobacterium* avium subsp. avium, *Mycobacterium* avium subsp. paratuberculosis, *Mycobacterium* ulcerans, and other mycobacteria are the aetiology of important diseases in humans and a wide range of animal species including, cattle, sheep, goats, elephants, poultry, and fish. Moreover, species such as *M. bovis* represent serious zoonotic pathogens and have become important agents at the interface of humans, domestic livestock and wildlife.

Relevance for food safety

Handling of contaminated animal products, including from aquatic sources reared under intensive conditions, may also lead to skin diseases in humans. In the case of marine animals, frequently observed species are *Mycobacterium* marinum and *Mycobacterium* fortuitum.

Final remarks

In humans and animals, the multi drug resistance potential is a serious concern in *M. bovis* and *M. tuberculosis*.

ZOONOTIC PROTOZOAN PARASITES

Zoonotic protozoan parasites are one-cell animal related to the protozoa group of protists animals.

Occurrence in feed

A multi-criteria based ranking for risk management of food-borne parasites by FAO/WHO has globally ranked several food-borne parasites by importance including an indication to their primary food vehicle as well as specific considerations for risk management at several points along the food chain.

Impact on animal health

Cryptosporidiosis is usually seen in calves between one and four weeks of age. It is very rare in animals older than a month because by this age most animals will have become immune to infection. The principal symptoms are diarrhoea, colic, reduced ingestion and weight loss.

Relevance for food safety

Humans could either act as an accidental host during the life cycle of the protozoa or an intermediate host in the life cycle of protozoa. *Giardia* of livestock typically does not seem to contaminate human host. *Cryptosporidium* found in horses, cattle, pigs, and sheep can accidentally infect humans causing diarrhoea. *Cryptosporidium parvum* and *Cryptosporidium hominis* are the two main species affecting humans. The top five parasites and food vehicles were noted as: *Taenia solium* in pork, *Echinococcus granulosus* in fresh produce, *Echinococcus multilocularis* in fresh produce, *Toxoplasma gondii* in meat from small ruminants, pork, beef, game (red meat and organs), and *Cryptosporidium* spp. in fresh produce, fruit juice, milk. Nevertheless, a clear transmission resulting directly from contaminated animal feed is less readily documented.

PRIONS

Bovine Spongiform Encephalopathy (BSE) is the main representative form of Transmissible Spongiform Encephalopathies (TSEs), caused by the presence of prions, which are, modified forms of host specific proteins. Scrapie is a related TSE affecting sheep and goats. The pathological form of the prion protein accumulates primarily in nervous system organs because of its resistance to proteolytic enzymes. The epidemiology and background of prions as a feed and food contaminant differs principally from those of chemical or (micro-)biological contaminations: prion diseases always have a progressive and irreversible nature, there is a genetic basis and resistance or cure does not exist.

Occurrence in feed

Elaborate analyses revealed that the most likely route for infection of susceptible animals is by oral ingestion of prions. These prions originated from certain ruminant animal by-products used as feed ingredients. Restrictions on feed vary from country to country, based on their own scientific risk assessments. The minimum recommended by the OIE is restrictions on feeding ruminant by-products to ruminants with exceptions that allow for feeding milk, tallow, and gelatine.

Several measures developed for inactivating prions at some stage in the feed and food production chain, such as composting, chlorine treatment and severe cleaning, heat treatment and acid or alkaline treatment appeared to be not fully effective. In the EU, measures against BSE are based on the following three principles (1) steam sterilization at 133° C, 3 bar and 20 min; (2) separation of animal by products into categories and adding a marker to those categories that must not added to feed; (3) introducing a permanent ban of animal by-products from ruminants from the feed chain; and (4) introducing a permanent ban of processed animal by-products in feed for ruminant.

Impact on animal health

Visual signs include change in temperament, abnormal posture, lack of coordination, decrease milk production and weight loss. The mechanism of transmission between animals is poorly understood. It is transmissible between individuals of the same species, and affected animals have to be killed and the carcass have to be destroyed to limit the contamination potential. Cattle and cows start to show symptoms at the age of four to five years, while scrapie affected sheep show symptoms from three to five years of age. Confirmation of suspected cases is possible mainly by histological direct analysis from brain samples collected from euthanized animals. International focus of surveillance now included the testing of targeted, high-risk cattle as the most effective way to detect BSE-infected animals.

Relevance for food safety

Consumption of animal products, mainly containing central nervous system parts, had been proven to be linked to cases of Creutzfeldt-Jakob disease. Ban on animal-based feed ingredients in most countries have significantly lowered positive cases in cattle and sheep and combined with strict inspection of herds has had a positive impact on infection rates. In the United Kingdom, no new case of Creutzfeldt-Jakob disease associated with beef consumption were observed after the ban.

Final remarks

Considering feed as the major vector for transmission, any control of feeding of wild animals suffering of Chronic Wasting Disease (CWD) is impossible.

Physical hazards

Physical hazards can be defined as those that can be introduced into feed via the feed manufacturing process and includes storage and transport processes. They do not transfer to animal tissues and as such should not be considered for food safety, but the migration of inks into edible tissues could occur.

These types of physical hazards can have a significant impact on animal health. Considerations are dependent on material, form and particle size as destined for particular animal species. Ruminants are not as sensitive to particles as would be chickens and young piglets. These should be controlled by Good Manufacturing Practices (GMP), GAP and HACCP.

RADIONUCLIDES

A physical hazard that may contribute to a food safety concern is Radionuclides. For the purposes of this document and consistency with Codex Alimentarius, these are being considered as physical hazards. Of specific importance are caesium-134, caesium-137, strontium-90, and iodine-131 present in animal feed and forages which may transfer to edible products. Major sources include contaminated soil, water and forage. Transfer of radioiodine to milk, radio-strontium to bone, and radio-caesium to milk, eggs and meat has been demonstrated. Considerations for a risk assessment include the half-life of the radioactive elements and its toxicokinetics. Radioactive iodine disappears in a relatively short time with a half-life of 8 days. Biological half-life of forms of caesium are longer than 60 days. Approximately 90 percent of radioactive caesium in feed consumed by animals is excreted in faeces and urine; the remainder is excreted in milk or remain in muscle. Radioactive substances distributed in muscles can be declined gradually on feeding cattle with non-contaminated feed.

NANOMATERIALS

Nanotechnology is defined by the International Organization for Standardization (ISO) as the “application of scientific knowledge to manipulate and control matter at the nanoscale in order to make use of size- and structure-dependent properties and phenomena, as distinct from those associated with individual atoms or molecules or with bulk materials” (ISO, 2010). Nanoparticles, or nanomaterials consisting of such particles, are generally accepted as those with a particle size below 100 nanometres.

Nanomaterials may be used as feed additives and its potential range is under investigation. Some examples include mycotoxin binders, delivery vehicles for trace elements and vitamins, and as a carrier for nutrients.

As a consequence of their small size, nanomaterials can exhibit different physicochemical properties and biological effects compared to their respective bulk materials. Very limited information is available on the potential transfer of nanomaterials from animal feed to edible products. There remains a lack of reliable characterization data of the nanomaterial in the product, and inadequate material characterization in the toxicological studies performed. The risk assessment of nanomaterials still heavily relies on animal studies. For the human safety assessment rodent species are used and no or only very few food producing animals have been used in toxicological studies. The potential transfer of nanomaterials from feed to edible products has not been studied so far.

Environmental contamination from the use of nanoparticles may result in inadvertent exposure to animals, including fish in aquatic environments. The physical properties of these particles may act as a carrier to increase the availability and exposure of other contaminants to animals.

MICRO- AND NANO-PLASTICS

High concentrations of plastic debris have been observed in the oceans. This is caused by commercial shipping, fishing and other activities in the oceans, but also due to increased release of micro- and nano-plastics through sewage or waste discharge that is caused by the increased occurrence of plastic particles in cosmetics, textiles, fishing nets, packaging and cleaning products over the last decades. Much of the recent concern has focused on micro-plastics. Micro-plastics are, because of their size (< 5 mm), not likely to be transported across cellular membranes, but as they might be present in the gut content, micro-plastic could end up in fish hydrolysates. It has been documented that microplastics can cause physical harm to aquatic animals and also be a vector of additives added to them during processing such as polybrominated diphenyl ethers (PBDE) and PCBs sorbed from the seawater to biota.

Nanoplastics include particles < 1 mm and two possible toxic effects are recognized for human health: the potential toxicity of the nanoplastic particles themselves, and the release of adhering Persistent Organic Pollutants (POPs) and leachable additives from these particles. Local effects on the gut epithelium (of environmental species but also humans) and the liver should be studied. The effects of nanoplastics on the gut epithelium may affect the barrier capacity of the gut wall also for other chemicals.

OTHER MATERIALS

Other physical hazards include glass, metal, sharps, paper and plastics. These can be introduced into feed via feed materials, feed ingredients, feed manufacturing and can be controlled or eliminated by sieving, and other means such magnets and metal detection.

Packaging materials are a typical consideration for all feed, especially for waste and/or by-products. They may be introduced into the feed manufacturing process via their inadvertent inclusion in feed from packaging of feed ingredients used in manufacturing. Seals, tags, or parts of the packaging itself sometimes fall into the mixer with the ingredients.

Former food to be used in feed manufacturing shall be unwrapped and free of packaging material as much as possible. In the case there is unavoidable material that remains in the former food, this should be of food grade.

Edible packaging materials represent an emerging system for food waste collection and storage. These are typically produced from starches, sugars or fibers which of themselves do not likely cause a safety concern. However, consideration should be raised for the inks, dyes, or other processing materials used in their manufacture.

Hazards of feed and products of feed production technologies of increasing relevance

Animal feed sometimes includes by-products produced by other industries. Such ingredients which now are considered traditional parts of an animal's diet are common – such as soybean meal, whey, fermentation by-products, seed hulls, and many others. However, new sources of "waste" from the food industries, biofuel industries and even industrial processes are increasingly being used. In addition, new types of ingredients such as insects, algae, krill, other marine resources and aquatic plants are contemplated for being used in feed.

These other sources of feed ingredients, however, can present new challenges to the risk assessment and management process.

Concerns with such new sources of feed, more so than with traditional ones, revolve around the clear identification and characterization of hazards which may be introduced through incoming materials that are used in the processing. Some new sources of feed can be generated from industrial manufacturing processes, or use waste products as feed for their production which can introduce new or higher concentrations of hazards not previously assessed in traditional feed, or also use new ingredients for which information is still limited. Thus, a new approach to evaluate them is needed. The safety of the new sources of feed can be determined by a three-step approach: identifying all incoming material used to produce the novel feed and their potential hazards; understanding the manufacturing process while identifying potential hazards introduced via processing; and a risk characterization of the final product itself. The evaluation of the new sources of feed should consider the role of manufacturing processes to mitigate the risk of the hazards.

Specifically, all steps of the manufacture of these "waste", by-products or new types of ingredients need to be considered including all processing aids used to treat or collect the material. For example, flocculants containing polyacrylamide polymers are sometimes used to collect solids, and additional fat or protein, from waste water streams. These often are then added back to the material for inclusion into the final feed.

The experts gave specific consideration to feed and products of feed production technologies of increasing relevance. Specific hazards and research requirements associated with the use of insects, former food and food processing by-products, biofuels (bioethanol and biodiesel) by-products, aquatic plants and marine resources as feed were highlighted as of primary importance. Methods of analysis, including multi-analyte methods, and sampling were also addressed and for each of the potential hazards both screening and confirmatory methods were considered.

INSECTS AS FEED

Dipteran fly larvae such as those from black soldier fly (*Hermetia illuscens*) and house fly (*Musca domestica*) contain up to around 63 percent protein and 36 percent fat (d.wt). They possess high levels of key amino acids (e.g. lysine, tryptophan) when compared to most crop plants, whilst possibly providing a safe, inexpensive and sustainable alternative to other animal products used in animal feed (e.g. fish meal, meat and bone meal). Fly larvae exhibit rapid growth and short life cycles (approximately 4-14 days). They can utilise a range of low value waste materials as feed and, particularly in the case of M. domestica, can tolerate a wide range of climates with relatively low requirements for land and light. For these reasons, insect use in feed is set to increase for natural insectivores (i.e. poultry, fish and pigs).

Insect producers in countries such as China, South Africa and the United States are already rearing large quantities of fly larvae for aquaculture and poultry feed using organic wastes, whilst smaller scale local farming of fly larvae in rural Africa is helping to reduce reliance on manufactured feed. Mealworms are also utilised for feed, possibly most notably in Thailand. Fly larvae derived products (protein, oil and fertilizer) may soon reach the scale required to become economically viable for wider industrial exploitation. However, there is currently a lack of data in the public domain relating to potential hazards associated with insect rearing for animal feed. There is also a lack of standardisation and guidance in relation to insect rearing practices at the international level.

Specific hazards with relevance for food safety

To date, studies on the microbiological and chemical safety of insects reared for feed are limited. Recently, review papers have been published on the microbiological and chemical safety of insects used for feed and food. Food safety authorities in the Netherlands and Belgium have published their opinions as has EFSA.

Ensuring that feed used for insect rearing does not contain or is unlikely to develop hazards that are passed through the food chain is the critical control point for ensuring risk reduction. Whilst downstream processing will largely manage microbial (and some chemical) risks, uncertainty remains in relation to persistent viral pathogens, which may be passively transferred initially in the insect gut via infected feedstock.

A recent study investigated the presence of a wide range of chemical contaminants including; residues of veterinary drugs and pesticides, mycotoxins, heavy metals, and dioxins in larvae of four different fly species, being the house fly, blue bottle (*Calliphora vomitoria*), blow fly (*Chrysomya* spp.) and black solder fly. The larvae were produced in different physical locations, with diverse rearing methods, using different waste materials. Levels of contaminants in the larvae were below recommended maximum concentrations permitted in other feeds. However, the toxic heavy metal cadmium was found to be of concern in three of the M. domestica samples analysed supporting data from other studies indicating possible heavy metal accumulation in wild insects.

Information about the possible transfer of substrate specific hazards such as prions from waste streams containing specified risk materials (e.g. abattoir, supermarket, restaurant or household food waste) and mycotoxins from e.g. contaminated or degraded cereal products, are missing at present. Allergenic risk in relation to animal health and occupational exposure during feed manufacturing is also of concern.

Research needs

Hazards associated with insects for use in feed depend on; feed, species, and production/processing conditions. To date, little information is publicly available about the hazards associated with insects for use in feed and this should be rectified through the adoption and sharing of best practise supported by robust analytical data for research and development (R&D) and regulatory purposes. Efforts to establish guidance in relation to standardisation of insect rearing and processing practises will also help to ameliorate risks, acknowledging that production scale and local requirements will have a significant influence on the approach taken.

FOOD WASTE AND FORMER FOOD PRODUCTS

There is a worldwide trend for waste reduction, including food waste reduction. This has led to an increase in the recycling and reuse of these products into the animal feed chain. While this may be considered normal agricultural practice, the increased exposure and the variety of food wastes becoming available can lead to a greater potential risk of emerging hazards in animal feed.

"Food Waste" can include materials that remain after, or are produced during, the processing, manufacture, preparation or sale of human food. This can include "Former Food Products", such as edible material intended for human consumption, arising at any point in the food supply chain, such as that collected at restaurants, retail, or from household food scraps.

"Food Processing by-products" include material that is recovered from food processing plants and may include some of the above listed material but also include production materials that are not intended as edible material.

It is important to stress that the animal feed chain should not be a means to dispose of degraded or contaminated foodstuffs, and that the product should have a nutritional value to be considered a feed.

Specific hazards with relevance to food safety

The hazard identification for former food products and food processing by-products is dependent on the product type and must consider the nature of the starting material, the processing steps to produce the original food item, the processing steps to produce the feed, and all handling, storage and transport steps. The re-introduction of any waste collecting processes, e.g. solids from wastewater treatments, filter cakes, cleaning materials, etc., should also be considered in the hazard identification step. Given the myriad the starting materials, considerations should be taken on a case by case basis.

Specific hazards from the manufacture of the final feed could include heavy metals, pesticides, dioxins and furans, mycotoxins, and residual processing aids.

Microbial hazards, shelf life and stability are potential concerns and relate to the relative moisture content of the end feed product. Due to the potential for concentrated bacterial growth, high moisture former food products and food processing by-products should be further heat treated.

Additionally, due to the lack of traceability associated with former food products there is the potential for an increased risk of animal products being fed back to animals. If foods of animal origin are included in the starting material, there may be risks for the transmission of animal diseases such as foot and mouth disease (FMD) and BSE.

Research needs

Risk assessment on the safe levels of packaging materials and inks/dyes contained in the food waste products is needed.

There needs to be increased communication between food and feed regulators and industries on the importance of the feed to food continuum and how to limit the diverting of contaminated food products to feed. The inclusion of quality control plans in food processing plants needs to extend to the safety of any end materials which may be diverted to feed and training should be provided to waste haulers and livestock producers to discuss the implications of safe handling and use.

BIOFUEL BY-PRODUCTS

The increasing demand for the environmentally friendly biofuels for the worlds expanding transport industry leads to increased use of raw materials for the production of biofuels. In many cases, this biofuel production yields by-products that may be used in livestock feed.

Bioethanol by-products

Distiller's dried grains with solubles (DDGS) is a high-protein feed from the bioethanol production. The process of producing DDGS starts by liquefying a crop, usually maize, wheat or sugar cane. Yeast is added to the mash and the product is subject to fermentation to produce ethanol. The resulting mash is distilled and centrifuged to remove liquids; the solubles that have been separated from the distillers grain in the liquid phase are concentrated by evaporation and re-added to the centrifuged solids before drying, producing DDGS that can be used for feed.

Specific hazards with relevance to food safety

A number of hazards have been reported related to biofuel and DDG process, of which the main ones include chemical hazards like mycotoxins, residual antibiotics and sulphate/sulphite.

Mycotoxins

If mycotoxins are present in the raw material used to produce ethanol and DDGS, they are not detoxified during the production process but instead, they are concentrated by a factor of approximately 3 compared to the raw cereal. Prevalent toxins include: aflatoxin, ochratoxin, fumonisins, deoxynivalenol, nivalenol, T-2, HT-2 and zearalenone. The mycotoxins can have multiple sources, including mould that has previously infected the crop in the field or in post-harvest stages. This is the main reason to reduce moisture content of DDGS. WDG (wet distiller´s grain), moisture content ± 65 percent as opposed to 10-12 percent for DDGS, has a much lower shelf life due to the growth of mould.

The impact on food safety depends on the transfer of the specific mycotoxins from the DDGS in feed to the animal products for human consumption. While most of the mycotoxins are metabolized and excreted by the animals with very limited transfer to the food, aflatoxin B1, the most toxic carcinogen of the mycotoxins is metabolized to aflatoxin M1 which occur in the milk. DDGS produced from crops from tropical or subtropical regions, e.g. maize is more likely to contain aflatoxin than DDGS produced from crops from temperate regions and in general the mycotoxin formation varies with climate conditions (see section 2.2.1 on mycotoxins).

Antibiotics

Some countries allow the use of processing additives such as antibiotics to be used to control the fermentation process by preventing bacteria growth. The antibiotics used in the production of biofuels, include virginiamycin, penicillin, erythromycin, tylosin and tetracycline. Of these virginiamycin is used most widely due to its approval for use in biofuel production in the United States In Europe, since 2006 antibiotics as additives for preventive purposes or for process improvement are banned in feed use due to risk of antimicrobial resistance. A study of biological activity of antibiotics used in fuel ethanol and corn co-product showed only activity in one of 159 samples of distillers grains (DDGS and WDG).

Sulphate/sulphite

During production of bioethanol, sulphuric acid is added during fermentation to keep the pH low and to keep the distillation columns clear of precipitate. Excess sulphur consumption (above 4 g/kg diet DM) from feed and water can lead to polio-encephalomalacia (PEM) and sulphur toxicosis; illnesses that may make livestock unsuitable for human consumption.

Biodiesel by-products

Glycerol is a by-product of the production of biodiesel via transesterification. Fatty acids released from oil and fats are esterified with methanol to produce biodiesel and water. Glycerol is separated from the crude biodiesel mash and residual methanol is partly removed.

The maximum methanol limit in glycerol for safe use in feed is 5000 mg/kg in the United States. The actual methanol level varies considerably per sample and may in some cases be toxic to ruminants. Regular practice is the addition of 10 percent of crude glycerol as dry matter in feed.

In the last decades, many plant species have been bred to produce biodiesel. The pressed cake that remains after oil extraction is often used for feed due to its high protein content. This is possibly hazardous to the animal as some of the plants cultivated for biodiesel contain highly toxic components. Curcin or jatrophin from *Jatropha curcas L.*, ricin from the castor oil plant (*Ricinus communis L.*), abrin from *Abrus precatorius L.* and croton from *Croton tiglium L.* are examples of hazards which may cause serious harm in livestock. Due to their high toxicity, chances of human ingestions through animals is minimal and when properly cooked these proteins will degrade rapidly.

Research needs

Currently, the crops used in fuel ethanol manufacturing are the same as those used as traditional feed sources. However, new techniques for the biofuel production has been developed which are based on raw materials containing cellulosic biomass such as leaves, wood or energy crops (e.g. switchgrass). Waste products from these alternative raw materials may also be used as feed ingredients. Finally, the utilization of algae biomass for biodiesel production may be a sustainable approach for biofuel production. The by-product formed from alage biomass using the new techniques may also be used for feed and require potential hazards to be assessed.

AQUATIC PLANTS

Aquatic plants are biologically different from algae as they are vascular plants that have adapted to living in aquatic environments (saltwater or freshwater). The reasons for using aquatic plants for animal feed purposes can be manifold, which include the following:

- Cultivation of free-floating aquatic plants generally requires few inputs for producing biomass. Recently, interest has increased for the cultivation and feed use plants such as duckweed (Lemnoideae family encompassing the *Landoltia, Lemna, Spirodela, Wolffia* and *Wolfiella genera*)
- Through uptake of minerals from the surrounding waters, such as agricultural and industrial waste streams, cultivated free-floating aquatic plants could be purposefully employed for water purification and recycling of minerals, including heavy metals, as long as threshold levels toxic to the plants themselves are not reached, as well as other minerals (e.g. phosphorus from faeces-containing water). Through removal of the minerals by the plants from these waste streams, the plants will concentrate these minerals, which will then be recycled via the use of harvested plants as fertilizer or feed. Moreover, they can be used to concentrate trace elements useful for animal nutrition. Aquatic plants such as duckweed are known to thrive in stagnant, nutrient-rich waters, such as those containing the excreta of other water animals. Under optimal conditions, the presence of nitrogen-containing compounds (particularly ammonia) induces high protein levels valued for animal feed purposes. Fertilization of aquatic plants can be done using inorganic fertilizers, y*et al.*so using manure under controlled nutrient in-flow conditions in ponds where these plants are grown. Based on these considerations, aquatic plants could fulfil a role in integrated farming, such as mineral recycling used for cleaning waste streams and recuperating minerals lost through e.g. run off from agricultural fields and returning these elements as feed or fertilizer to the farm
- Conversion of invasive or otherwise nuisance-causing prolific, wild aquatic plants, such as water hyacinth (*Eichhornia crassipes*) mats physically blocking and de-oxygenating waterways to biomass.

Specific hazards with relevance to food safety

Aquatic plants tend to take up and concentrate minerals from the surrounding water. Moreover, they may be used purposefully for removal of particular minerals from waste streams such as heavy metals, in which case the potential hazard of these metals occurring in the harvested aquatic plant should be verified.

As aquatic plants have applications for purification of wastewater streams, particularly including those containing animal manure and human faeces (e.g. sewage), the presence of faecal, pathogenic microorganisms, particularly the zoonotic ones that can transfer from feed via animal food products, should be checked for.

Depending on environmental conditions, aquatic plants may contain residues, such as pesticide residues in case of systemic uptake of pesticides from surface waters or contaminants such as polycyclic aromatic hydrocarbons (PAH), a class of combustion by-products with toxicological properties that raise concerns as several PAH are genotoxic carcinogens and/or have dioxin-like (endocrine disrupting) properties.

Research needs

While duckweed (particularly Lemna in ecotoxicity testing) and several other aquatic plants have been and still are well-investigated for their potential to take up and metabolize hazardous compounds/minerals and biological hazards, this may not be true for other aquatic plants for which interest as potential feed ingredient has arisen. The collection of sufficient adequate data on the potential occurrence of hazards (accumulated from the environment such as the ones discussed above) should be investigated.

Some aquatic species are considered for cultivation while others will probably also be harvested from the wild (e.g. as part of eradication), raising concerns of the lack of control over exposure of the latter to environmental feed hazards throughout their lifecycle. The safety of such plants collected in the wild should be corroborated with adequate data.

As alternative feed ingredients, plants have limitations such as the presence of antinutrients (phytates, tannins) and imbalanced fatty acid profiles; these features might impact adversely on feed.

More research is needed to assess the significance for consumer safety of PAH transfer, including the characterization of specific PAHs present in edible fish tissues and the potential intake through fish fed alternative plant-derived feed materials in the context of the overall PAH dietary intake. Also, research is need in order to understand the factors (plant species/cultivars, feed processing, etc.) that modulate the PAH content.

More research is also desirable on the composition of plant-derived ingredients that will minimize any potential hazard for fish health and productivity.

MARINE RESOURCES

Feed ingredients from marine resources are intended for aquaculture; currently, no novel marine sources are intended for use in feed for land animals. Traditional aquaculture feed consists mainly of fishmeal and fish oil, processed from fish specifically caught for feed purposes. However, due to fishing quotas and environmental issues, the amount of fish caught for feed cannot keep up with demand from the steady increase in aquaculture production.

Fish hydrolisates from fishing bycatch and/or fish waste

Fishing activities have a significant output of side-products that are not used for human food:

i) bycatch: this includes non-relevant species or juvenile specimen that are not suitable for sale. The portion of bycatch that is transported back to land can be processed and reutilized as feed. The use of bycatch appears as worth developing as an environmentally friendly practice; however, it is noted that an unregulated increase would contribute to overfishing and further pressure on fish stocks;
ii) fish parts in waste water from fish processing plants.

Both ingredients are hydrolysed and used as a source of aquaculture feed.

Specific hazards with relevance to food safety

Due to the intensive protocol to produce fish hydrolysate, no major microbiological hazards are expected, provided that the feed material is properly stored.

Fish hydrolysate is expected to present chemical hazards (e.g. methylmercury, dioxins and other POPs) comparable to the more conventional feed materials, fish meal and fish oil. Differences in the concentrations of contaminants may be related to composition of feed ingredients, in terms of dry matter, protein and fat.

The use of the ocean bycatch may introduce nano- and microplastics in the feed hydrolysate as a hazard: both kinds of particles are significantly present in marine, and especially ocean, environments as a result of plastic debris produced by shipping and fishing activities as well from environmental release of plastic particles from packaging and consumer products. Indeed, trophic transfer of nano- and microplastics does take place from the marine environment through to fish and fish hydrolysates. However, the two kinds of contaminants are quite different.

Microplastics have a size > 1 µm, thus, absorption in the fish organism and intracellular bio accessibility are very low or absent; however, they may end up in fish hydrolysates due to their presence in the gut content. Microplastics are unlikely to have any transfer from feed to edible fish tissues; however, they might pose a hazard to fish production and health by impairing the fish digestive function. Pollutants, e.g. heavy metals, may adhere to microparticles and be carried into the feed.

Nanoplastics might be bio accessible; a transfer from feed to food cannot be excluded. The small size (in particular when below 100 nm) entrains two characteristics of potential concern: a) the ability to enter into the cells and interact with macromolecules, including DNA, with toxicological effects still to be properly investigated; b) the nanoparticles have a relatively wide surface, thus are able to catch and transport other molecules, including toxic contaminants such as POPs.

Research needs

The available data do not allow a proper risk assessment of the presence of microparticles and nanoparticles in fish hydrolysates. The highest priority has to be given to nanoparticles, for which both transfer to consumers and toxicological properties need to be characterized. The biological behaviour, including toxicology, of nanoparticles is related to the dose and the specific material, but also to the physical characteristics (particle size, surface shapes, and aggregation status); these characteristics should be measured for a proper toxicological assessment. Therefore, analytical techniques to detect nanoparticles must be developed and standardized, in order to assess the exposure of feed, fish and consumers.

Krill

The macroplankton crustacean populations called krill can be a valuable source of aquaculture feed, due to their high availability in the Antarctic and North Atlantic sea as well as nutritional properties, such as the high content of omega-3 fatty acids.

Specific hazards with relevance to food safety

Krill organisms are at the bottom of the food web, thus they are not expected to undergo a major bioaccumulation of toxic contaminants such as methylmercury or PBDE. Indeed, the replacement of conventional fish meal with krill meal has led to 80-96 percent lower concentrations of arsenic and mercury in salmon flesh.

However, more data may be desirable on the potential of krill to absorb hazards, (e.g. pathogenic microorganisms, toxic elements or nanoplastics) from the water medium.

The shell of krill contains relatively large amounts of fluoride compared to conventional fish feed. A high fluoride content is clearly undesirable; however, the hazard should not be overemphasized: fluorine does not concentrate in edible fish tissues but only in the fish skeleton, thus the transfer to consumer would be minimal. Moreover, the farmed fish species have a low susceptibility to fluorosis, contrary to some mammalian species, such as cattle. If needed, the excess fluorine can be markedly reduced by removing the exoskeleton before krill is processed into feed.

Research needs

More data are needed on the krill potential to absorb and retain biological hazards, chemical contaminants as well as micro- and nanoplastics from the water medium; i.e. more data are desirable on the influence of the water environment on the krill composition, including the presence of biological or chemical hazards.

Algae

Algae are a large and biologically diverse group of non-flowering plants growing in fresh water and marine environments. For the purpose of this document, the three main species groups are brown, red and green algae: whereas current utilization of algae is primarily as food, applications in the feed field are developing. A different group, blue-green algae, can produce a series of toxins and have currently only a limited application in the food field.

Algae may be used directly as feed ingredients; in addition, a use as biomass for biofuel production is envisaged. This might lead to another indirect presence of algal material in feed, i.e. as biofuel by-products.

Specific hazards with relevance to food safety

Algae may concentrate many potentially toxic elements, including arsenic, chromium, cadmium, lead and manganese. In many marine algae arsenic appears to be present in the form of arsenosugars, which are considered of low toxicity compared to inorganic arsenic; however, at least one algal species, *Sargassum fusiforme*, shows a marked potential for accumulation of inorganic arsenic. The content of potentially toxic elements depends on the algal species, as well as on the medium on which algae are grown. The potential for transfer to food of these toxic elements depend on the concentration, the chemical species as well as the ability of the element to bioconcentrate in the farm animal species consuming the algae-based feed.

Algae can have a high content of iodine. Several essential trace elements (e.g. selenium and iodine) can have a significant toxicity at intakes above requirements in humans; thus, attention should be given to feed materials that are particularly rich in such trace elements. In particular, excess iodine in feed may be a concern for consumer safety, due to the transfer to milk and eggs. Moreover, the excessive enrichment of algae-derived feed materials with some trace elements (zinc, manganese, iodine, etc.), may lead the complete feed to exceed the legal limits set in some countries/areas besides safety issues, to be considered separately for each element, this may create obvious problems to the usage and marketability of algae-based feed materials.

Micro-algae, including toxin-producing blue-green algae, can unintentionally be harvested in mixed cultivation; thus, macro-algae might act as vector for accumulated marine toxins. As for freshwater algae, green algae can uptake from their environment and accumulate microcystins, a toxic product of certain freshwater cyanobacteria. Good control of the cultivation environment can prevent the contamination by toxins. Some marine algae have a significant content of lectins, which can be markedly toxic for farm animals, e.g. pigs. It is advisable not to use species with a high lectin content.

Research needs

More information is needed about the potential of different algae to accumulate toxic elements (including excessive concentrations of essential elements), the speciation of such elements when relevant (e.g. inorganic vs. organic arsenic) and the influence of environmental and cultivation conditions.

It is also desirable to have information on the conditions influencing the accumulation of toxins in algal species and the potential transfer of toxins from feed to edible tissues and products.

Plant ingredients in fish feed

Fish meal and fish oil from feral fish are widely used as feed ingredients, particularly in fish feed.

Beyond stricter control and monitoring of feed for aquaculture, the use of alternative ingredients in fish feed has been envisaged. Besides feed contamination, pressure on feral fish stocks, and consequently limited access to fish meal and fish oil for the rapidly growing aquaculture sector has contributed to the development of aquaculture feed based on alternative ingredients such as plants.

Specific hazards with relevance to food safety

The replacement of fish oils with plant oils has been shown to decrease dioxin and PCB levels in farmed salmon by 50-80 percent compared with fish raised on feed based on fish oil (Sprague, M. 2010). Moreover, the combined use of plant oils and krill meals achieves an overall reduction of both methylmercury and POPS compared to the conventional aquaculture feed.

In contrast, PAH levels were significantly higher in fish raised on alternative feed due to the higher PAH concentrations in these feed matched with the PAH potential for transfer and their apparent inefficient metabolism in fish on animal health and production as well as on the nutritional profile of fish flesh.

Research needs

The use of plant ingredients in fish feed is a very promising approach to drastically reduce the dietary exposure of consumers to contaminants; in particular, the use of plant oils may achieve a great reduction of the presence of POPs in fish for human consumption.

OTHER NEW SOURCES OF FEED INGREDIENTS (DERIVED FROM INDUSTRIAL BY-PRODUCTS)

The diversion of wastes from industrial processes to animal feed is increasing. This can represent the inclusion of previously not assessed processes, industrial grade processing aids and new contaminants. The detailed manufacturing process must be considered in order to identify any hazards which may carry forward into the final feed ingredient. As an example, many mineral sources for feed are now recouped from other industrial processes such as lime from kiln dust, reclaimed copper from circuit boards and batteries, zinc oxide from waste sites, etc. A second example includes the production of new sources of fatty acids from the further processing of residual material after the production of a vegetable oil. A third example is a new source of cellulose derived from the pulp and paper processing of wood.

Specific hazards with relevance to food safety

These new processes may introduce unacceptable residues of heavy metals, dioxins, furans, PCBs and new processing chemicals into the final feed ingredient. This needs to be assessed on a case-by-case basis after a careful assessment of the complete manufacturing process.

Research needs

Better communication on these new processes through collaboration between industry and regulators will better capture ingredients in a transparent manner.

Innovation utilizing waste streams needs to be developed in a safe manner with an understanding that the end use must be suitable for introduction into the food chain.

Methods of analysis and sampling

METHODS OF ANALYSIS

When analysing samples for compliance check against target levels, methods are often classified whether they are used for screening or confirmatory purposes. In the table below for each of the potential hazards both screening and confirmatory methods are listed that are used in practice, in other words that have proved their reliability. It should be stressed that most of these methods have not been validated for all relevant feed ingredients and feed.

As is shown in Table 3 no reliable methods are available for a number of the hazards, notably for nanomaterials. This indicates the strong need for development of these methods.

Table 3: Selected examples of screening and confirmatory methods that are currently used in practice

Group of hazards	Screening methods		Confirmatory methods	
	Methods available	Remarks	Methods available	Remarks
Dioxins + dl-PCBs	Bio-assays (Calux) (Hoogenboom *et al.*)	Multi-analyte	GC/HRMS, GC-MS/MS (European Commission, 2014)	Multi-analyte
Mycotoxins	Dipsticks, ELISA (Maragos *et al.*., von Holst et Stroka)	Also multi-analyte	LC-MS/MS, LC-FLUO (Stroka *et al.*, Mol *et al.*)	Also multi-class together with plant toxins
Heavy metals			AAS, ICP-MS (EN 15510)	LC-ICP-MS for inorganic arsenic and methylmercury; AAS for inorganic arsenic after SPE pre-separation
Veterinary drugs	Dipsticks, ELISA (Borras *et al.*)	Also multi-analyte	LC-MS/MS (Kaklamanos *et al.*)	
Organochlorine pesticides	GC-ECD		GC-MS	
Plant toxins	Dipsticks (tropane alkaloids), (Mulder *et al.*) ELISA (pyrrolizidine alkaloids) Oplatowska *et al.*	Also multi-analyte; for many plant toxins screening methods are not yet available	LC-MS/MS(Mol *et al.*)	Also multi-class together with mycotoxins; For some of the emerging plant toxins no methods are available
Brominated flame retardants			GC-MS/MS, LC-MS/MS (for PBDEs) Lankova *et al.*	GC-MS/MS: Also together with dl-PCBs, ndl-PCBs, PAHs

(cont.)

Table 3 *(cont.)*

Group of hazards	Screening methods		Confirmatory methods	
	Methods available	Remarks	Methods available	Remarks
Pathogens	Conventional PCR (Jarquin *et al.*)		Culture-based assays (MPN), real-time PCR and qPCR, (Okele and Fink-Gremmels)	Disadvantage of qPCR: pre-enrichment necessary to distinguish between viable and non-viable counts
Parasites			Microscopy	
GMO's	PCR (both for authorised and unauthorised GMOs) Dipsticks (for some GMOs) Protein based methods (for some GMOs) (Scholten *et al.*)		Real-time PCR or qPCR (for known GMOs, either authorised or unauthorised) (European Union Reference Laboratory for GM Food and Feed)	
Animal proteins	Dipstick, ELISA, NIR-Microscopy (Raamsdonk *et al.*, Boix *et al.*)		Microscopy, PCR, mass spectrometry (Fumière *et al.*, Flaudrops *et al.*)	Microscopy can discriminate between terrestrial and fish material but cannot discriminate further. PCR: false-positive results if milk constituents are present. Mass spectrometry: differentiation between not banned feed materials such as milk powder and banned processed animal proteins is possible
Packaging materials			Visually, microscopy (Raamsdonk *et al.* 2012)	
Microplastics			Microscopy (Raamsdonk *et al.* 2012)	
Radionuclides			gamma-ray spectrometry measurements (Desideri *et al.*)	
Nanomaterials	Not available yet		Not available yet	

Abbreviations: AAS: Atomic Absorption Spectroscopy; ECD: Electron Capture Detection; GC: Gas Chromatography; ELISA: Enzyme-linked immunosorbent assay; LC: Liquid Chromatography; ICP: Inductively Coupled Plasma; HRMS: High Resolution MS; MPN: Most Probable Number; MS: Mass Spectrometry; MS/MS: tandem MS; NIR: Near Infrared; PCR: Polymerase chain reaction; qPCR: quantitative PCR; SPE: Solid-phase Extraction

SCOPE OF ANALYSIS

The purpose of this chapter is to elaborate on the main characteristics of screening and confirmatory methods. In addition, the aspect of multi-analyte methods is explained.

Screening methods

There are various reasons why screening test are applied under real world conditions. The most important reasons are that many of these methods neither require laboratory environment nor highly educated personnel. Moreover, the screening methods are less expensive and often the duration of the analysis is shorter compared to conventional methods.

These methods are typically applied in situations, where a high number of samples needs to be analysed and there are some indications that the majority of samples is below this target level. Screening tests are designed to identify samples exceeding this level. Negative samples are accepted as such. Positive samples need to be subjected to confirmatory analysis when measuring within the frame of official control, because screening methods may lead to false positive results. In other situations, for instance for quality control or when advanced methods are not available, a positive result may directly trigger an action without further measurements.

The impact of false positive results on the measurement exercise needs to be evaluated case-by-case considering also other factors. For instance, if there is very high cost reduction by using screening tests compared to conventional methods, an increased rate of false positive results can be accepted.

Screening methods are often only validated for frequently used feed ingredients such as cereals.

It should be noted that when these tests are used for complex products such as compound feed, the performance may be worse. For this reason, screening methods should always be validated for each product of concern to see if the method is fit for the purpose.

Confirmatory methods

Samples that are flagged as positive could undergo a confirmatory test to check for compliance with the target level. Confirmatory test requires the use of more sophisticated methods that have been thoroughly validated, for instance by conducting a collaborative trial. However, the latter requires considerable planning in terms of design of the trial, the type of matrix or matrices to be analysed, the level of chemical hazard and the number of samples.

Multi-analyte methods

Multi-analyte assays are methods enabling the detection of multiple analytes in one test. The objectives for the development of multi-analyte assays are as simple and quick sample pre-treatment alternatives with high-throughput screening settings for the analysis of large numbers of samples on-site at low cost. To date, there is increased efforts toward the development of multi-analyte assays focused on different analytes, such as enzyme-linked immunoassays, fluorescence immunoassays, and other immunoassays based on different solid supports or biosensors for the detection of pesticides, electroanalytical sensors and devices for the detection and identification of pathogen microorganisms, polymerase chain reaction techniques for the detection of viruses, bacteria and other pathogen microorganisms, liquid chromatography-tandem mass spectrometry for the determination of mycotoxins, photoinitiators and amine synergists in food and foodstuffs. These rapid and sensitive multiplex assays could satisfy the requirements of other environmental materials detection and quantification.

METHODS OF SAMPLING

The need to obtain a representative sample deserves special attention since a wrong sampling plan can greatly affect the reliability of the measured levels.

Sampling is one of the steps that contribute to the variability of analyses due to nonhomogeneous nature of most chemical hazard distribution in food and feed. Sampling for chemical hazard analysis is still a challenge especially in developing world where resources (fund, specialized human capital) are lacking. There are some guidelines and regulations regarding sampling. However, the implementation of these regulations in most developing countries (e.g. Africa) is sometimes difficult due to insufficient/lack of resources.

Because sampling plays critical role in the reliability of the measured levels of chemical hazard contaminating food and feed a proper and adaptable sampling strategy is required. Therefore, there is need for developing or revision of the existing sampling guides.

Alternative schemes sampling should be evaluated against the probability of taking the right/wrong decision.

There is need for the development of appropriate/dedicated sampling procedure for local trade.

Conclusions and recommendations

CONCLUSIONS

Highlighting that hazards in feed may present an important risk for human health as a result of transfer from feed to food of animal origin, and can have a negative impact on animal health and welfare, the meeting stressed the importance of pursuing the prevention and control of hazards in animal feed. Standards, guidelines and practical measures to ensure safe feed need to be developed and implemented, at both national and international levels. Action from multiple players is required to build upon what has already been done to address feed safety by Codex, FAO, WHO and other organizations, national regulators and the feed industry. Ongoing and enhanced capacity development is an important aspect of improving feed safety, particularly in the context of changing feed production systems and feed sources, the need for sustainability in animal production systems and the broader context of global food security.

The expert meeting highlighted the role of risk assessment as well as the numerous challenges in undertaking risk assessment presented by the wide range of hazards and feed sources, including the need to generate the necessary data on some of these contaminants, collate that data, (if feasible through a global platform and where necessary develop the methodologies needed to facilitate such risk assessment. For example, sampling approaches and sampling plans were identified as a key area to be addressed in terms of data collection and monitoring of hazards in animal feed. The role of the industry in generating data to facilitate risk assessment as well as that of national authorities and international bodies to ensure that such data are generated was emphasized.

While not explicitly addressed, the expert meeting was keen to emphasize that the value of available data and risk assessment is only realized when subsequent risk management measures are identified and implemented and noted that the information provided in this report serves as a starting point in focusing risk management action.

Noting the recognition that Codex Alimentarius gives to safe feed for the production of safe food, the meeting concluded that in order to provide countries with the tools they need to manage feed safety, there was now a need for Codex to continue including explicit consideration of feed when developing or revising Codex standards, codes of practice and other relevant texts for biological and chemical contaminants. The meeting also recognized the differences that exist between countries in relation to their regulatory frameworks for feed, in particular between high-, middle- and low-income countries, and the impact this can have on the potential to manage such hazards. This may be a particular issue in many low-income countries where legislation and infrastructure for the management of feed safety is still immature or even non-existent. The ongoing development of new technologies to make use of available potential feed sources in the context of increasing demand for foods of animal origin highlights the importance of having capacity to address, not only the assessment aspects, but also drive the development of institutional frameworks.

While regulatory aspects could not be addressed within this meeting they were highlighted as important issues to be considered by feed regulatory fora.

The meeting did not attempt to prioritize the hazards that were reviewed as it concluded that this should be undertaken on a country-by-country basis taking into consideration the specific situation, including feed sources and production systems and the guidance on prioritization of hazards in feed developed by Codex prioritization process. The meeting underlined food security as an additional criterion to consider. Overall however, the meeting highlighted that in the changing environment in which feed is now being produced and used, whether it be changes in climate, farming practices or the increasing use of different feed sources and feed production technologies, there is a need to regularly review the potential hazards from these feed sources, to be aware of the potential for new hazards to emerge and be ready to take the necessary steps to manage these.

While genetically modified organisms (GMOs) were addressed in the meeting, given the increasing adoption of this technology in crops used as feed worldwide over the last two decades, it was also considered that the products of GMOs are not hazards as such, as each one should be subject to an assessment for safety prior to use in line with Codex Guidelines for the Conduct of Food Safety Assessment of Foods derived from Recombinant DNA Plants.

Finally, the meeting recognized the breath of information on analytical methods for detection of hazards and the challenges it presents for countries in terms of both accessing that knowledge and understanding what is relevant for animal feed. For example, many of the existing methods for hazard detection have not been validated for all relevant feed and feed ingredients while no reliable methods are available for a number of the identified hazards. In this context, the expert meeting developed a table of information to provide users with an overview of the methods available specifically for hazards in feed and the scope of their application, which serves as a unique reference point for such information.

CONCLUSIONS ON SOME OF THE HAZARDS CONSIDERED

The range of potential hazards associated with feed is broad and possibly increasing with the rising importance of different feed sources and feed production technologies. Many of these hazards are relevant irrespective of the feed source but the local production environment and the specific production processes can be critical in terms of their prevalence.

Chemical hazards

Persistent organic pollutants

POPs are ubiquitous and bioaccumulate in the lipid rich tissues of animals. In particular, the expert meeting considered polychlorinated dibenzo-dioxins (referred to as dioxins from here on), dl-PCBs and ndl-PCBs.

Dioxins are a group of contaminants with common toxicity pathways; the reproductive, immune and endocrine systems are sensitive targets, especially in developing organisms. Thus, dioxins are a priority hazard for feed and food safety.

The ubiquitous presence of dioxins and dl-PCBs in the environment from both natural and anthropogenic sources contributes to their potential presence in feed. Elevated environmental levels have been associated with soil and plant material on flood plains in industrial areas and also with soil and plant material in areas close to sources of industrial emissions. Fishmeal and fish oil produced using fish harvested from contaminated areas can also contain relatively high levels of dioxins. Industrial sources of contamination have included ball clay used as an anticaking agent in feed, lime as a neutralizing agent for citrus pulp, contaminated oils, some mineral sources and most recently contaminated fatty acids. Direct drying of feed, using inappropriate fuel, is another potential source of dioxins. Addressing the food safety risks posed by dioxin and dl-PCBs in feed, requires information on the lipid content of the feed and on the congener profile of these hazards in the feed, which impacts their transfer from feed to food. Dioxin and dl-PCBs are only slowly eliminated and as such levels found in edible tissues, and milk and eggs, are dependent on the levels in feed and also the duration of exposure.

It is difficult to identify particular adverse effects in humans and animals of ndl-PCBs due to the co-occurrence of the more toxic dioxins and dl-PCBs. Effects on liver, thyroid, reproduction and neurodevelopment have been reported in association with different congeners or congener groups. Work needs to continue internationally to better define the risk associated with these compounds that are generally present at much higher levels in feed than dioxins and dl-PCBs. Ndl-PCBs accumulate in fat, liver, fillets of oily fish and are also transferred to lipid-rich products like milk and eggs. There are differences in the uptake, metabolism, accumulation and excretion as well as toxicity of the different ndl-PCB congeners.

Organochlorine and other pesticides

Organochlorines are persistent, lipophilic compounds that behave much like dioxins and PCBs and are recognized contaminants of fats (e.g. fish oils) used in feed. On the other hand, less attention has been paid to the potential of other pesticide groups to contaminate feed and transfer to foods of animal origin. Transfer to animal products, metabolism and toxicity of specific pesticides used in plants intended for feed production should be examined prior to pesticide authorization and the establishment of MRLs for feed and foods of animal origin. The expert meeting noted that existing authorization mechanisms and established MRLs may not always reflect the extent of all plant products that may end up in feed. Additionally, if these plant products are subject to processing, residues may concentrate in by-products that are used as feed.

Veterinary drug residues

Feed remains a much-used vehicle for the efficient delivery of veterinary drugs to animals. While transfer, metabolism and toxicity of veterinary drugs in feed to animal products is fully assessed as part of the authorization process and establishment of MRLs, the expert meeting noted that this does not cover the different non-target species which may be exposed via cross-contamination of feed, and this may be an important consideration for risk management in some countries.

The issue of veterinary drug residues in feed and food has long been recognized due to long standing concerns for public, animal and environmental health as a result of direct exposure to these residues and concerns that residues of antimicrobials may be associated with the development of antimicrobial resistance.

Potentially toxic elements (PTEs)

While arsenic, cadmium, lead, mercury, selenium, copper, nickel and chromium are natural components of earth materials, they also have an anthropogenic origin. They can be toxic for animals and transfer to the animal products may occur. For a number of these elements transfer from feed to foods of animal origin tends to be low due to low absorption (e.g. inorganic arsenic, lead) but for others where the half-life is long, e.g. cadmium, significant levels can accumulate, for example in crustaceans. Accumulation of inorganic cadmium in livestock depends on the level and duration of exposure. Methylmercury is a specific, and widely recognized, problem for aquaculture feed based on fish-derived meals.

Mycotoxins

Mycotoxins contaminate farming systems globally. When ingested in high concentrations through feed derived from plant material they exert severe toxic effects in animals, may decrease their productivity and may accumulate in edible tissues and animal products, resulting in human exposure and health effects. Besides Aflatoxin M1 (a carcinogenic agent) in milk and ochratoxin in meat, milk and eggs, the other well-known mycotoxins e.g. zearalenone, fumonisins may seriously affect animal health and productivity, but do not show a major transfer to foods of animal origin. However, the profile of mycotoxins of importance continues to evolve. There are a range of known toxins that are likely to change in relative importance with evolving agricultural practices, including the use of different feed sources. Moreover, there are likely to be many as yet unrecognized mycotoxins, given that there are many thousands of fungal species, each producing many secondary metabolites that have not been assessed for toxigenicity. This presents an ongoing challenge for monitoring, risk assessment and risk management and in this context the meeting highlighted the importance of mitigating mycotoxin contamination along the feed chain using a range of measures and tools relevant to the local situation. As it is very difficult to remove mycotoxins from contaminated feed, preventing them from accumulating in agricultural commodities is the most effective strategy to combat the problem. Preventive measures range from crop rotation and resistance breeding to inoculation with microbial antagonists and storage management. Continuous monitoring is essential and efficient detoxification strategies are needed to deal with outbreaks and the risks posed by low level exposure.

Plant toxins

Toxin-producing plants may occur in grasslands used in forage and are a significant cause of livestock poisoning. Transfer of some of these toxins to edible products such as eggs, milk and meat has been demonstrated, for example in the case of genotoxic pyrrolizidine alkaloids. Changes in toxin occurrence in plants and concentrations of plant toxins may be caused by climate changes and worldwide an increased occurrence of some toxin producing weeds has been observed which results in a spread of the accompanying risks. Also changes in farming practices from migratory herds to expanded settlement and crop cultivation in dry season grazing land can mean that animals have access to a reduced variety of plants and thus potentially greater exposure to toxic plants. Addressing this means that efforts are needed to decrease toxicity and anti-nutritional factors in existing and newly available feed. Given the variety of toxic plants, this presents extensive challenges for risk assessment and further data is needed to accurately characterize this type of hazard and the dose–effect relationship.

Other potential and emerging chemical hazards

A range of contaminants including brominated flame retardants and perfluorinated compounds have been shown to be present at low levels in animal feed. However, the expert meeting noted that there is currently insufficient information to assess whether the transfer via feed of these compounds presents a risk to human and animal health.

Biological hazards

There is a continuous risk for contamination of feed by microbial pathogens throughout the production chain up to feeding to the animals, with the many opportunities for contamination making it difficult to control or fully eliminate specific pathogens. A key aspect of managing this risk is the identification and characterization of pathogens of concern in feed understanding, among other things, the factors that affect the sources, and routes of contamination. *Salmonella* has been identified as an important hazard and a wide spectrum of *Salmonella* serovars have been isolated from feed. This includes those most commonly isolated from clinical cases of human salmonellosis, like *Typhimurium* and *Enteritidis*. Other potential bacterial hazards in animal feed to be aware of, according to the feed type include *Mycobacterium*, *Brucella*, *Clostridium* spp., enterohaemorrhagic *Escherichia coli* and *Listeria.* Parasites in pasture and forage have also been identified as potential hazards. Viruses were also considered and it was noted that in terms of those that are a concern for food safety and human health there is little evidence to date of feed as a source of their transmission to food. However, it was also noted that as feed sources and processing technologies change there is a need to be aware of the impact of these on biological hazards. The lessons learned with the emergence of prions in the not too distant past should not be forgotten. In terms of emerging issues, it was also noted that managing environmental diffusion of these hazards will require better knowledge of survival of those organisms under environmental stresses (temperature, irradiation, and desiccation) and climatic changes, which are influencing farm practices, may also present opportunities for increased prevalence and risk from biological hazards.

Physical hazards

Physical hazards, except nanomaterials and radionuclides, do not transfer to animal tissues and as such were not considered in terms of food safety; however, they can have a significant impact on animal health. Considerations are dependent on material, form and particle size as destined for particular animal species, for example, ruminants are not as sensitive to particles as would be chickens and young piglets. However, this is an area where there are some emerging issues, e.g. packaging materials due to use of former food products in animal feed, micro- and nano-plastics in marine environments and therefore the meeting concluded that the relevance of physical hazards in feed for food safety should not be dismissed.

Hazards of feed and products of feed production technologies of increasing relevance

There is an increasing interest, production and use of certain feed ingredients such as insects, former food and food processing by-products, biofuels (bioethanol and biodiesel) by-products, aquatic plants and marine resources. These can present new challenges for risk assessment and management. Concerns revolve around the clear identification and characterization of hazards that may be introduced through incoming materials that are used in processing. In this context, a new approach to evaluate the safety of such feed is needed: identifying all incoming material used to produce the feed and their potential hazards, understanding the manufacturing process while identifying potential hazards introduced via processing, and a risk characterization of the final feed product itself. The evaluation of the feed should consider the role of manufacturing processes to mitigate the risk of the hazards. The meeting considered the following feed production technologies and sources that are of increasing relevance:

Insects as feed

To date, there is little information in the public domain about the hazards associated with insects for use in feed and this is an important gap to be addressed for regulatory purposes. Efforts to establish guidance in relation to standardization of insect rearing and processing practices will help to ameliorate risks, acknowledging that production scale and local requirements will have a significant influence on the approach taken.

Food waste and former food products

The worldwide trend for food waste reduction has led to an increase in the recycling and reuse of former food and food processing by-products in the feed chain, which, if not well managed can lead to a greater potential risk of emerging hazards in animal feed. While this can be an important pathway for waste conversion, it is critical from a safety perspective that the feed chain is not used as a means to dispose of degraded or contaminated foodstuffs. Given the diversity of inputs, the range of hazards relevant to feed from these sources could be very broad ranging from heavy metals, pesticides, dioxins and polychlorinated dibenzo-furans, mycotoxins, and residual processing aids, packaging materials, particularly from former food products, and microbial hazards, which can increase particularly in high moisture former food. With the lack of traceability associated with some of these inputs, particularly in the case of post-consumer waste, the expert meeting highlighted that there is potentially an increased risk of animal products being fed back to animals and cycling of hazards in the feed-food chain. The expert meeting noted the need for increased communication between food and feed regulators and industries on the importance of the feed to food continuum and how to limit the diversion of contaminated food products to feed.

Biofuel by products

Biofuel production yields by-products that may be used in livestock feed. These include dried distiller's grains with solubles (DDGS), a high-protein feed from the bioethanol production and the high protein pressed cake and glycerol that remains after oil extraction and processing for biodiesel. A number of hazards have been reported related to these processes including mycotoxins, residual antibiotics and sulphate / sulphite. Also some of the plants cultivated for biodiesel can be toxic to animals. This presents a new challenge in terms of working with an industry outside of the traditional feed/food sector to ensure that the risks associated with these by-products potentially entering the food chain are minimized.

Aquatic plants

Aquatic plants are of increasing interest as feed due to the low inputs required to produce biomass. However, these plants also often serve other purposes such as water purification and can take up and concentrate minerals and other potential hazards from their environment. Microbiological hazards may also be a concern due to growth of the plants in waste water. Whether these plants are cultivated or harvested from the wild there is clearly a need for further research.

Marine resources

Algae may be used directly as feed ingredients and as their use as biomass for biofuel production is also envisaged, this might lead to another indirect presence of algal material in feed, i.e., as biofuel by-products. The expert meeting noted that algae may concentrate many chemical elements, including toxicologically relevant ones (e.g. arsenic, chromium, cadmium, lead) and essential elements that can be toxic at excess doses (e.g. iodine) depending on the algal species, as well as on the medium on which algae are grown, and transfer to food of animal origin will be dependent on a number of factors including ability to bioconcentrate in the consuming species. Another aspect is the potential to unintentionally harvest toxin producing micro algae with algae intended for animal feed in which case algal feed materials can be vectors for accumulated marine toxins. More information is needed about the potential of different algae to accumulate toxic elements, the speciation of such elements when relevant (inorganic vs. organic arsenic) and the influence of environmental conditions, as well as the conditions influencing the accumulation of toxins in algal species and the potential transfer, if any, of toxins from feed to edible tissues and products.

As fishing activities have a significant output of side-products such as by-catch and fish parts from fish processing plants these can be hydrolyzed and grained as aquaculture feed. Chemical contamination of these hydrolysates was considered to be comparable to conventional fish feed but increased levels of nano- and micro-plastics may be an issue.

Krill can be a valuable source of aquaculture feed and may lead to lower exposure to conventional hazards associated with aquaculture feed such as mercury. In terms of specific hazards, krill may contain relatively large amounts of fluoride compared to conventional fish feed but if needed the excess fluorine can be addressed by removing the exoskeleton before processing into feed.

RECOMMENDATIONS

In light of the above conclusions, the expert meeting recommended:

- FAO and WHO to develop guidelines for the prevention and control of hazards identified in feed to support the efforts of member countries in addressing these hazards;
- FAO, WHO and Member Countries and their capacity development partners to continue with and further enhance capacity development activities, especially on risk assessment and management of hazards in feed, including for feed sources and technologies of increasing relevance, to better meet domestic and international standards.

Need for international standards

- The Codex Committee on Contaminants in Food and the Codex Committee on Food Hygiene to develop and update specific provisions for the control and reduction of chemical and biological contaminants in feed, including decontamination measures, for inclusion in Codex codes of practices for prevention and reduction of food and feed contaminants;
- The Codex Committee on Food Hygiene to address the issues of biological hazards re- entering the food chain through the development of specific guidance on the control of recycled foodstuffs, including food processing by-products and former food, through feed, particularly in the context of the Codex General Principles of Food Hygiene;
- The Codex Alimentarius Commission to revise and update the Codex Code of Practice on Good Animal Feeding to address new hazards derived from the use of feed and products of feed production technologies of increasing relevance;
- The Codex Committee on Pesticide Residues and Member Countries to establish MRLs for pesticides of concern in feed;
- Member Countries to encourage the Codex Committee on Contaminants in Food to establish MRLs for contaminants of concern in feed.

With regard to feed sources and technologies of increasing relevance to the feed sector:

- FAO, WHO and Member Countries to raise awareness within the food industry, including food retailers, on the importance of maintaining safety standards of former food and/or food processing by-products, when these products are reused as feed;
- The food industry to extend the quality control measures in food processing plants to identify hazards in by products/waste when they will be recycled into feed;
- The food and feed industry to provide information and data on processing aids used during food and feed processing in order to identify any hazards that may be transferred to feed through the use of processing aids;
- FAO and WHO to develop guidelines for the safe production and use of insects as feed;
- FAO and WHO to develop guidelines for the safe production and use of biofuel-by-products as feed;

- FAO and WHO to develop guidelines for the safe production and use of feed from former food products and food processing by products;
- The feed industry, Member Countries, FAO and WHO, possibly in collaboration with the World Organisation for Animal Health (OIE) to identify hazards in feed and assess their impact on food safety and animal health due to the recycling of animal products e.g. fish by-products back into fish feed, animal fat into feed.

To support risk assessment of hazards in animal feed:

- FAO and WHO to collect, through the extension of the Global Environment Monitoring System (GEMS/food), monitoring data regarding the occurrence in feed of the hazards described in this report;
- Member Countries, FAO and WHO to encourage regulators to require relevant data packages from industry to support the risk assessment of pesticides residues in feed. Consider pesticide residues in feed especially as they pertain to feed ingredients, including by-products used as feed, which may not have been previously assessed;
- FAO, WHO and the Codex Committee on Residues of Veterinary Drugs in Food to consider the risk from the residues of veterinary drugs resulting from cross-contamination from medicated to non medicated feed in international risk assessment and where necessary develop the appropriate risk management measures;
- The Organisation for Economic Co-operation and Development (OECD) and FAO to identify specific considerations relevant to the assessment of feed and products of feed production technologies of increasing relevance and where relevant update existing guidance and tools which would facilitate international harmonization in this area;
- FAO and WHO to develop guidelines for appropriate sampling of feed recognizing that hazards in feed are not always homogeneously distributed;
- Member Countries to develop risk-based approaches/procedures and sampling plans for data collection and monitoring of hazards in feed.

Research needs

- The scientific community and Member Countries to carry out further research to identify, characterize and prioritize potential hazards from environmental pollutants, and determine their occurrence in feed;
- The scientific community to carry out research and FAO and WHO to develop methods/guidelines for risk assessment on the combined effects on human and animal health of multiple hazards of particular relevance to feed;
- The scientific community and Member Countries to carry out further research to identify, characterize and prioritize plant toxins, with particular attention to the development of analytical methods, and determine their occurrence in feed;
- The scientific community and the industry assess hazards in raw materials and other substances used for production of biofuel when by-products are diverted to feed;
- The scientific community to carry out research to improve the identification and characterization of hazards in feed sources of increasing relevance such as insects and aquatic plants and marine products and resources;

- The scientific community to carry out scientific research and FAO and WHO promote a harmonized approach for risk assessment of hazards associated with nanomaterials in feed and nanoparticles present in the environment, which may contaminate feed. This includes the development of analytical methods for the determination and characterization of nanoparticles in feed.

Future work be undertaken

- Because of the short duration of the expert meeting, many important issues that were within its scope could not be considered in detail. Continued discussion is required at an international level to allow addressing more compressively the following:
- Hazards in feed that are of particular concern for animal health and productivity, taking into consideration the need to ensure food security;
- Other risks to human health, such as occupational health issues, and when feasible their inclusion in feed risk assessment.

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CODEX TEXTS RELEVANT TO ANIMAL FEEDING

Standards

General Standard for Contaminants and Toxins in Food and Feed (CXS 193-1995) (melamine)

Codes of practices

Code of Practice for the Reduction of Aflatoxin B_1 in Raw Materials and Supplemental Feedingstuffs for Milk-Producing Animals (CXC 45-1997)

Code of Practice for Source Directed Measures to Reduce Contamination of Food with Chemicals (CXC 49-2001)

Code of Practice for the Prevention and Reduction of Mycotoxin Contamination in Cereals, including Annexes on Ochratoxin A, Zearalenone, Fumonisins and Tricothecenes (CXC 51-2003)

Code of Practice on Good Animal Feeding (CXC 54-2004)

Code of Hygienic Practice for Meat (CXC 58-2005)

Code of Practice to Minimise and Contain Antimicrobial Resistance (CXC 61-2005)

Principles for Traceability/Product Tracing as a Tool within a Food Inspection and Certification System (CXC 60-2006)
Code of Practice for the Prevention and Reduction of Dioxin and Dioxin-like PCB Contamination in Food and Feeds (CXC 62-2006)
Code of Practice for Weed Control to Prevent and Reduce Pyrrolizidine Alkaloid Contamination in Food and Feed (CXC 74-2014)

Guidelines

Guidelines for the Exchange of Information in Food Control Emergency Situations (CXG 19-1995)
Guidelines for the Exchange of Information between Countries on Rejections of Imported Foods (CXG 25-1997)
Guidelines for the Production, Processing, Labelling and Marketing of Organically Produced Foods (CXG 32-1999)
Methods of Sampling for Pesticide Residues for the Determination of Compliance with MRLs (CXG 33-1999)
Analysis of Pesticide Residues: Guidelines on Good Laboratory Practice in Pesticide Residue Analysis (GXG 40-1993)
Guidelines for the Design and Implementation of National Regulatory Food Safety Assurance Programmes Associated with the Use of Veterinary Drugs in Food Producing Animals (CXG 71-2009)
Guidelines on the Application of Risk Assessment for Feed (CXG 80-2013)
Guidance for Governments of Prioritizing Hazards in Feed (GXG 81-2013)

MRLs

Maximum Residue Limits and Risk Management Recommendations for Residues Of Veterinary Drugs in Foods (CXM 2) (online database: http://www.fao.org/fao-who-codexalimentarius/codex-texts/dbs/vetdrugs/en/)
Maximum Residue Limits (MRL) for Pesticides (CAC/MRL 1) (online database: http://www.fao.org/fao-who-codexalimentarius/codex-texts/dbs/pestres/en/)

Miscellaneous

Classification of food and animal feed (CXM 4)

APPENDIX
Background document

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Introduction

A rapidly growing population, along with an increase in urbanization and income are driving the demand for foods of animal origin, both terrestrial and aquatic. The demand for animal products is estimated to be possibly 70% higher in 2050, compared to today. Concurrently, the demand for animal feed, including aquafeed will continue to go up with an increase in the food-feed-fuel competition and in food prices. Measures to produce food and feed more efficiently and to reduce food and feed losses and wastes are necessary to face this challenge. The challenge is not only to meet the growing demand for feed but also to ensure its safety in terms of (i) food safety, through prevention and control of transfer of hazards to food of animal origin and (ii) animal health. Feed is an integral part of the food chain and its safety has been recognized as a shared value and a shared responsibility.

Hazards may be introduced directly through their presence in feed ingredients, feed additives and water for drinking or via cross-contamination or formation during production, handling, storage, transportation and feeding of feed ingredients and feeds. The presence of a hazard may also result from accidental or deliberate (e.g. fraud) human intervention. Hazards associated with feed can be of a chemical, biological or physical nature and include among others mycotoxins, heavy metals, dioxins, PCBs, residues of veterinary drugs and pesticides, plant toxins, pathogenic microorganisms, prions and remnants of / undesirable substances present in packaging materials. New hazards may be associated with feed ingredients of increasing relevance which are entering the production chain, e.g. agro-industrial by-products (such as those of the biofuel industry), industrial by-products such as reclaimed minerals, insects, former food products, marine products (such as krill) and algae. Production technologies of increasing relevance such as nanotechnology and genetic modification may also lead to the introduction of new hazards.

The Codex Alimentarius Commission (CAC) adopted the Code of Practice on Good Animal Feeding (CXC 54-2004) in 2004. (FAO, WHO, 2008a). The CAC has also adopted in 2013 Guidelines on the Application of Risk Assessment for Feed (CXG 80-2013) (FAO, WHO, 2013a) and Guidance for Governments on Prioritizing Hazards in Feed (CAC/GL 81-2013) (FAO, WHO, 2013b). After completing work on these two documents, the Codex ad hoc Intergovernmental Task Force on Animal Feeding, noting the availability and on-going emergence of new information on hazards in feed of relevance to human health, requested FAO and WHO to update the findings of the 2007 FAO/WHO Expert Meeting on Animal Feed Impact on Food Safety (FAO, WHO, 2008b).

This review paper has been prepared by RIKILT Wageningen University & Research on request of FAO and WHO to serve as the background paper and basis for discussion at the Joint FAO/WHO Expert Meeting on hazards associated with animal feed, Rome, 12-15 May 2015.

The objective of the Expert Meeting was to provide Member Countries with an updated overview of the current state of knowledge on hazards associated with both conventional feed and feed ingredients as well as feed ingredients and products of feed production technologies of increasing relevance. The meeting also provided guidance on the most appropriate use of this information for risk analyses purposes, identified knowledge gaps and prioritized future work on the identification of potential hazards of key global concern from the perspective of human and animal health.

Prior to and during the meeting, experts and international organisations (International Feed Industry Federation, IFIF) were invited to comment on the content of the background document. The comments and suggested references are included in the final version of this background document as far as relevant. The relevance was thoroughly discussed and agreed by a review committee consisting of FAO officials and RIKILT Wageningen University & Research - authors of this background document.

The review paper includes the results of a literature search, covering the period from January 2007 to January 2015. In chapter 2 the literature search protocols are described. Moreover, flow-diagrams are included that contain criteria to qualify the potential impact on human and animal health and the potential transfer to food of animal origin of the hazards described in chapter 3 as high, medium or low and the strength of evidence for these qualifications (strong, moderate or weak). In chapter 3 new insights in hazards of conventional feed and feed ingredients are reviewed. In some cases (notably for plant toxins and mycotoxins), the potential hazards of rangelands and pastures grazed by domestic livestock have also been included. In chapter 4 hazards of several types of feed ingredients and products of feed production technologies of increasing relevance (nanotechnology and biotechnology) are described. In chapter 5 new developments in analytical methods for the detection of hazards, including rapid methods and multi-analyte methods, are reviewed.

This review covers the potential hazards related to feed for livestock animals, fish and other seafood species, such as shellfish. Pet food and bee feed are not included in this review. Animal feed includes complete and complementary feed (e.g. mineral feed) and feed ingredients (also often referred to as feed materials), including feed additives and premixtures. In this paper, according to Codex-definitions (Codex 2004), feed and its constituents will be specified as “feed and feed ingredients”.

It is important to note that the exposure to certain hazards is heavily dependent on the diets for specific animal species, which in their turn depend on the respective digestive physiology and nutritional requirements. As an example, ruminants require much more roughage compared to monogastric species, whereas fish require high concentrations of fats and proteins. A distinction is made between (i) conventional feed and feed ingredients (Chapter 3) and (ii) feed and feed ingredients of increasing relevance (Chapter 4). Conventional feed and feed ingredients include among others, cereals, oilseeds, food processing by-products that are already used for a long period as feed ingredients (e.g. soybean meal, maize gluten feed, palm kernel expellers), forages, fishmeal, fish oil, minerals and feed additives. Feed and feed ingredients of increasing relevance include insects, former food and food processing by-products, biofuel-by-products, marine products (fish hydrolysates and krill), algae and products derived from production technologies of increasing relevance, viz. genetic modification, synthetic biology and nanotechnology. While some of these feed ingredients, e.g. insects and former food products, have already been used for many years in certain regions of the world, the choices to specify them as “of increasing relevance” may be disputed. The main reason behind these choices is that these feed ingredients / technologies were not included in the 2007 FAO/WHO-review (FAO, WHO, 2008b).

In this paper, the potential hazards associated with animal feed, including feed for aquaculture, are reviewed, based on a literature search for the period from January 2007 to January 2015. Where relevant, older reports and review papers, e.g. from FAO, WHO, Codex, FDA or EFSA were also included. In this final version of the background document, many references have been included that were suggested by the experts and moreover, review papers and documents published by international organisations during the period 2005 – 2007 are included.

Hazards related to food safety, through transfer from feed to food of animal origin, and animal health are covered. Also, the role of feed in the development of antimicrobial resistance is reviewed. Potential sources of hazards are described, including feed ingredients, feed additives and water for drinking and hazards introduced via cross-contamination and formation during production, handling, storage, transportation and feeding of feed ingredients and feeds. The review includes chemical, biological and physical hazards, viz. dioxins and dioxin-like PCBs, non-dioxin-like PCBs, organochlorine pesticides, potentially toxic elements, brominated flame retardants, perfluorinated compounds, mycotoxins, plant toxins, residues of veterinary drugs, pathogenic microorganisms, antibiotic resistance, viruses, prions, endoparasites and remnants of / undesirable substances present in packaging materials. Regarding veterinary drugs, only the hazards related to cross-contamination levels in feed and other indirect routes of contamination of feed ingredients are evaluated. Medicated feed is excluded from the review. For nearly each section a summary table was prepared that summarizes information regarding sources of contamination, transfer of hazards to food of animal origin, potential impact on human and animal health, strength of evidence and knowledge gaps.

While it is acknowledged that representative sampling of feed ingredients can greatly affect the reliability of the conclusions regarding the contamination levels of lots, especially for contaminants that are heterogeneously distributed in a lot, this topic is not within the scope of this report. Safety of workers in feed mills and storage facilities is also outside the scope of this report.

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Methods

LITERATURE SEARCH

Databases

In order to provide the experts authoring the various sections on topics within their fields of expertise with recent, supplementary literature references from scientific literature, searches were performed in four different databases known to extensively cover the fields of life sciences, in particular agriculture, veterinary sciences, toxicology, nutrition and analytical sciences, namely AGRIS (FAO), CAB Abstracts, Scopus, and Web of Science.

Search strings

To this end, search strings were employed for a range of specific topics, including the various types of chemical, microbiological, and physical hazards, as well as various novel types of feed ingredients (Table 1). The search strings consisted of words associated with the particular topic, combined with the use of Boolean operators ("and", "or") and proximity operators (words within a specified distance from each other). Given that FAO/WHO concluded a previous review on the topic in 2007, this year was taken as starting year for the publication date of the references (i.e., a limiting criterion, published after 2006). In addition, the search was limited to publications in English given that foreign-language publications might not be accessible to authors given possible language barriers.

For the different types of hazards and topics, adjusted search strings were used with regard to breadth and scope, and level of search (i.e., title only or title & abstract & keywords), as follows:

- **Chemical hazards:** multiple strings to cover the wide diversity of chemical hazards
  - For some chemical hazards (e.g. mycotoxins, plant toxins) the strings would already contain hundreds or even thousands of terms, which would make it arduous for experts to process and summarize this information. Particularly for these voluminous datasets, a more restrictive search was additionally carried out so as to obtain a more limited set of references with still a high proportion of references to relevant publications. Using the same search strings, a two-step approach was thus followed, namely:
    - An **extensive** search. In this search, the occurrence of all search terms within a string was checked for in title, abstract and keywords (i.e., the option "all fields" in CAB/AGRIS, "topic" in Web of Science" and "TITLE-ABS-KEY" for title, abstract and keywords in Scopus).
    - In the more **restrictive** searches, the same search terms were used yet a part of these were only searched for in the title (instead of title & abstract & keywords) while the other terms within the same string were also searched for in abstract and keywords. For example, for search strings focusing on a chemical or biological hazard, the hazard-related terms (e.g."dioxins") were looked for in the title only, whereas the terms related to animal feed (e.g. "livestock adj4 feed" were searched for in all fields, and

the outcomes (of these substrings) were then combined using the "and" operator to retrieve records for references on the particular hazard (dioxins) occurring in animal feed. This way, it was anticipated that a lower number of records would be retrieved than in the extensive search, while these were more likely to have the hazard or feed ingredient of interest as a main feature in the article since these were explicitly mentioned in the title. For several hazards and ingredients, the extensive search already yielded a limited number of references, such as on the relatively new topic of krill, so that the restrictive search outcomes are only for information purposes and the more extensive search outcomes were considered as the major information source.

- For **biological hazards** occurring in animal feed (including among others microbiological hazards, viruses, parasites, and prions): A single search string was used, both in extensive and restrictive mode similar to chemical hazards,
- For **novel feed ingredients**, ingredient-specific search strings were developed and used. Similar to chemical and biological hazards, both extensive and more restrictive search procedures were applied. In the restrictive search, it was the ingredient-related terms (e.g., "krill") that were looked for in the title only (instead of the title & abstract & keywords targeted in the extensive search), while the terms related to hazards and risks were searched for in all fields.

Search outcomes

In Table 1, the outcomes of the "extensive" and "restrictive" searches are summarized. The searches would yield a number of records containing bibliographic data (e.g., title of the document, year of publication, authors' names and journal title), abstract, and possibly a link to its original full-text version.

Processing of results

The records thus obtained from the various databases were transferred and combined for each topic within a file operated by the reference management program Endnote, which was made available to the authors of this paper. In addition, authors of the paper had been provided with a mini-protocol, so as to provide guidance on how to screen the Endnote files for relevant references. In the protocol, inclusion or exclusion of references based on their relevance was recommended to be done through a tiered approach. The first step entailed browsing the titles in the Endnote file, screening them for relevance. The criteria for judging whether a reference is relevant included:

- The reference should pertain to animal feed
- Either the presence or absence of hazards, risks, or adverse effects in livestock animals and/or cultured fish and other aquaculture species should be reported
- The hazard/risk/effect should be "new" either because it occurs in a new animal feed or because it was not previously conceived to be such in existing animal feeds

Of records with titles that appeared to be relevant indeed, or of which the relevance could not be determined, it was recommended checking the abstract for confirmation. Authors could thus establish lists of selected references from which they could draw information for their summary review of the particular topics that they had been assigned to. Besides using the data thus retrieved, it was realized that authors could also take advantage of the data from additional sources, such as Codex Alimentarius standards and other FAO/WHO documents, pertinent books and other sources deemed relevant by the authors based on their expertise and experience in the particular field of interest.

Table 1: Topics and sub-topics covered by dedicated search strings used for searching four databases for relevant references on hazards in animal feeds

Topics & sub-topics	Records retrieved		Selected publications
	Extensive search	Restrictive search	
Developments in hazards specifically identified by FAO/WHO (2007) and by authors			
Biological hazards:			
- Biological hazards	979	75	15
- Antimicrobial resistance	672	21	10
Chemical hazards:			
- Brominated flame retardants	252	10	2
- Dioxins and dioxin-like PCBs	514	202	18
- Heavy metals	3,708	210	16
- Mycotoxins	2,680	283	23
- Non-dioxin-like PCBs	13	5	3
- Organochlorine pesticide residues	321	19	10
- Plant toxins	1,287	56	27
- Veterinary drug residues	523	71	5
Specifically identified, unconventional feed and feed ingredients:			
- Algae	282	16	13
- Biofuel production by-products	149	41	7
- Food waste	137	20	5
- Genetically modified organisms & synthetic biology	211	34	6
- Herbs, botanicals	342	16	19
- Insects	213	11	4
- Marine protein, krill	37	2	8
- Nanotechnology	35	1	31
New analytical technologies to measure chemical, biological, and physical hazards			
Analysis of hazards:			
- Chemical hazards	175	(n.a.)*	18
- Biological hazards	226	(n.a.)	12
- Physical hazards	6	(n.a.)	2

* (n.a.) = not applicable

CRITERIA TO QUALIFY THE POTENTIAL IMPACT OF HAZARDS AND STRENGTH OF THE UNDERLYING EVIDENCE

The summary tables at the start of each section on a particular chemical or microbiological hazard define the magnitude of the potential health impact of the hazard on humans and animals, and its transfer from feed to food of animal origin. Also the strength of the evidence underlying these qualifications is indicated. For the qualification of these impacts and strengths, criteria were applied according to a decision-tree approach, for which the flow-diagrams displayed below were used.

For the description of health impact on animals and humans, the factors considered pertain to severity of health effects, measures already imposed to manage the risk, and the hazard's agility to proliferate. The three impact categories thus assigned are high, medium, and low. In more detail, these criteria consider whether the hazard:

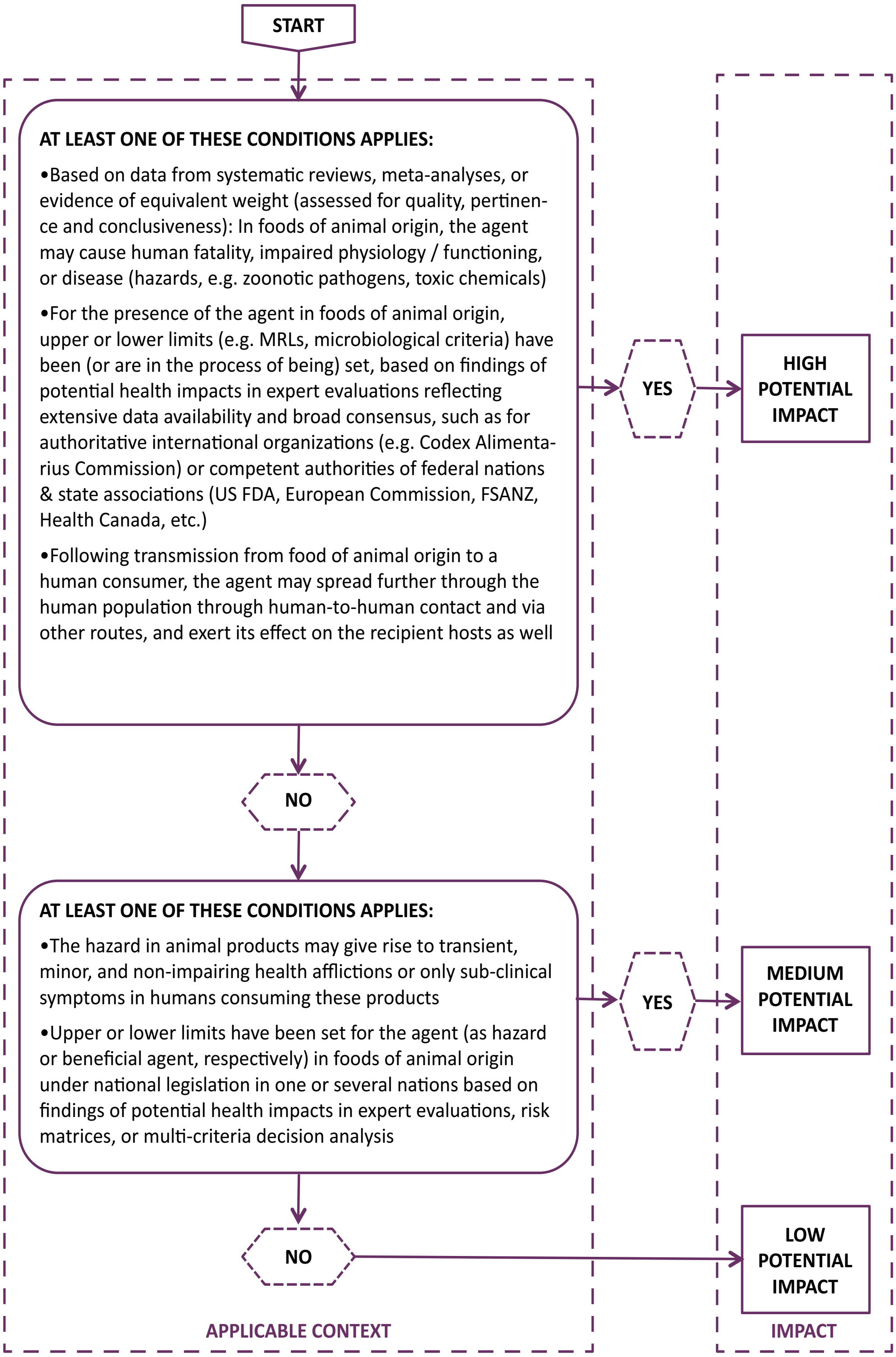
- causes severe health effects, such as fatality or permanent disability, as opposed to transient, minor health afflictions
- has already been subject of risk assessments and measures and standards have already been imposed in many nations/internationally in order to manage the associated risks
- can rapidly proliferate throughout a population following exposure of a member of that population to the hazard

With regard to the potential impact of feed-to-food transfer, the criteria consider whether the hazard

- is transferred to a significant degree according to scientific data
- has already been risk-assessed and subject to regulatory limits in feed so as to prevent exceedance of safety thresholds in food after transfer.

For the concept of "strength of evidence", it is important to consider how resilient certain outcomes are to changes in variables and how conclusions can stand up to alternative explanations. In foodborne disease outbreaks, the strength may rely on epidemiological associations between a given pathogen or toxicant and disease in consumers, as well as on molecular evidence linking the occurrence of a particular pathogen strain or toxicant in food to this disease. Also mechanistic studies elucidating the mode of action of a hazard can add to the plausibility of an adverse outcome pathway. Three levels were chosen for the classification of strength of evidence, namely strong, moderate, and weak.

Potential impact on human health



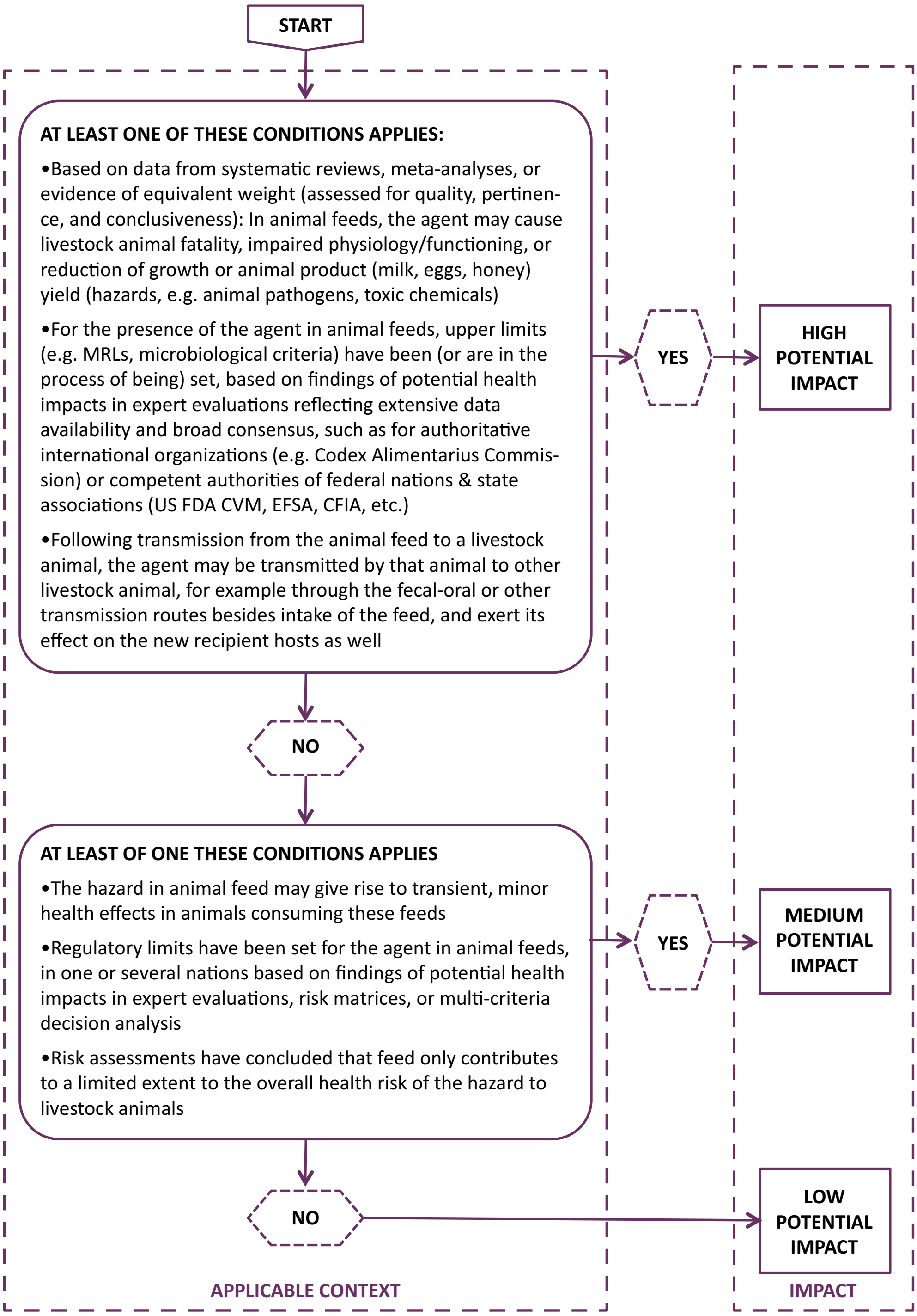
AT LEAST ONE OF THESE CONDITIONS APPLIES:

- Based on data from systematic reviews, meta-analyses, or evidence of equivalent weight (assessed for quality, pertinence and conclusiveness): In foods of animal origin, the agent may cause human fatality, impaired physiology / functioning, or disease (hazards, e.g. zoonotic pathogens, toxic chemicals)
- For the presence of the agent in foods of animal origin, upper or lower limits (e.g. MRLs, microbiological criteria) have been (or are in the process of being) set, based on findings of potential health impacts in expert evaluations reflecting extensive data availability and broad consensus, such as for authoritative international organizations (e.g. Codex Alimentarius Commission) or competent authorities of federal nations & state associations (US FDA, European Commission, FSANZ, Health Canada, etc.)
- Following transmission from food of animal origin to a human consumer, the agent may spread further through the human population through human-to-human contact and via other routes, and exert its effect on the recipient hosts as well

AT LEAST ONE OF THESE CONDITIONS APPLIES:

- The hazard in animal products may give rise to transient, minor, and non-impairing health afflictions or only sub-clinical symptoms in humans consuming these products
- Upper or lower limits have been set for the agent (as hazard or beneficial agent, respectively) in foods of animal origin under national legislation in one or several nations based on findings of potential health impacts in expert evaluations, risk matrices, or multi-criteria decision analysis

Potential impact on animal health



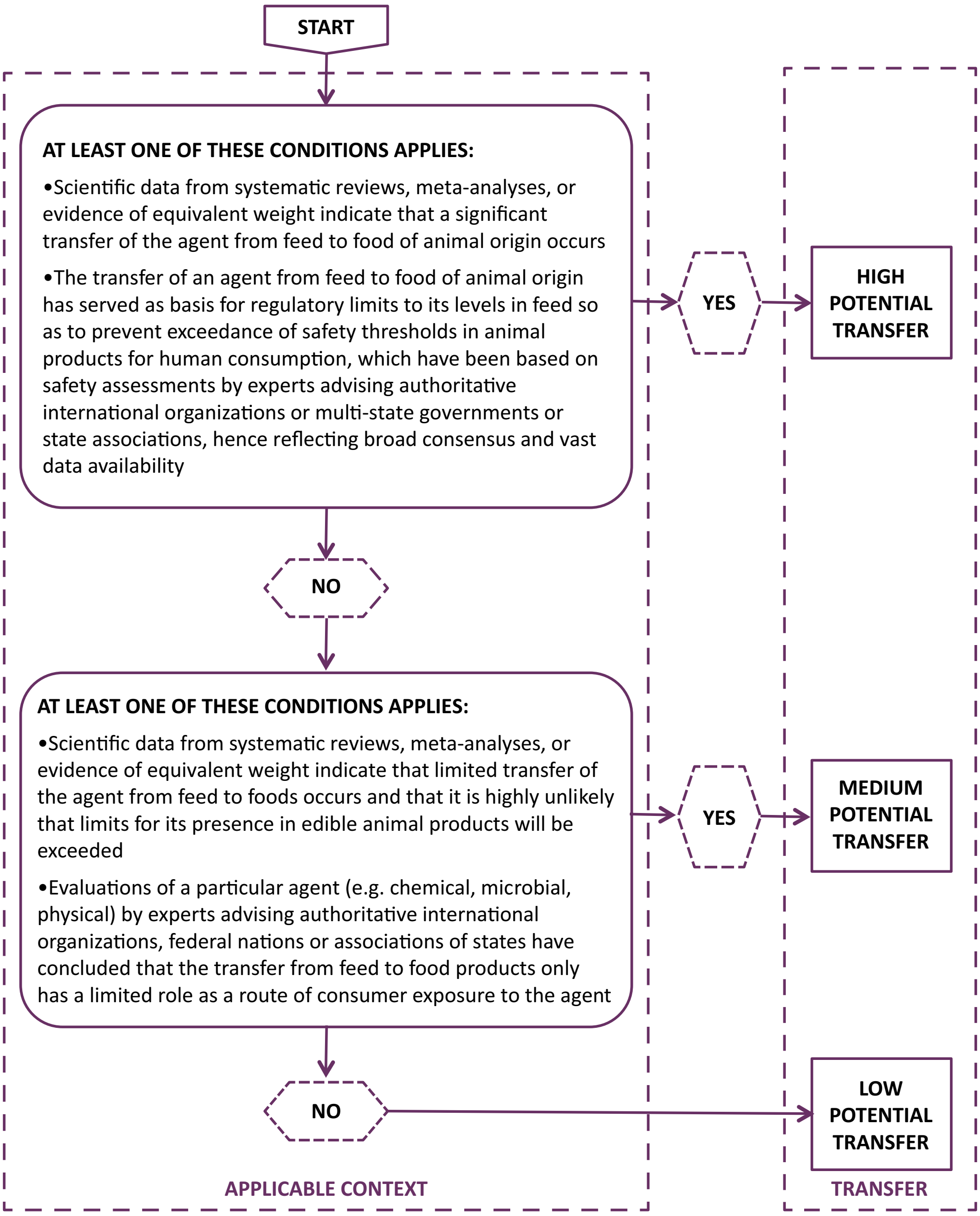
AT LEAST ONE OF THESE CONDITIONS APPLIES:

- Based on data from systematic reviews, meta-analyses, or evidence of equivalent weight (assessed for quality, pertinence, and conclusiveness): In animal feeds, the agent may cause livestock animal fatality, impaired physiology/functioning, or reduction of growth or animal product (milk, eggs, honey) yield (hazards, e.g. animal pathogens, toxic chemicals)
- For the presence of the agent in animal feeds, upper limits (e.g. MRLs, microbiological criteria) have been (or are in the process of being) set, based on findings of potential health impacts in expert evaluations reflecting extensive data availability and broad consensus, such as for authoritative international organizations (e.g. Codex Alimentarius Commission) or competent authorities of federal nations & state associations (US FDA CVM, EFSA, CFIA, etc.)
- Following transmission from the animal feed to a livestock animal, the agent may be transmitted by that animal to other livestock animal, for example through the fecal-oral or other transmission routes besides intake of the feed, and exert its effect on the new recipient hosts as well

AT LEAST OF ONE THESE CONDITIONS APPLIES

- The hazard in animal feed may give rise to transient, minor health effects in animals consuming these feeds
- Regulatory limits have been set for the agent in animal feeds, in one or several nations based on findings of potential health impacts in expert evaluations, risk matrices, or multi-criteria decision analysis
- Risk assessments have concluded that feed only contributes to a limited extent to the overall health risk of the hazard to livestock animals

Potential transfer to food of animal origin



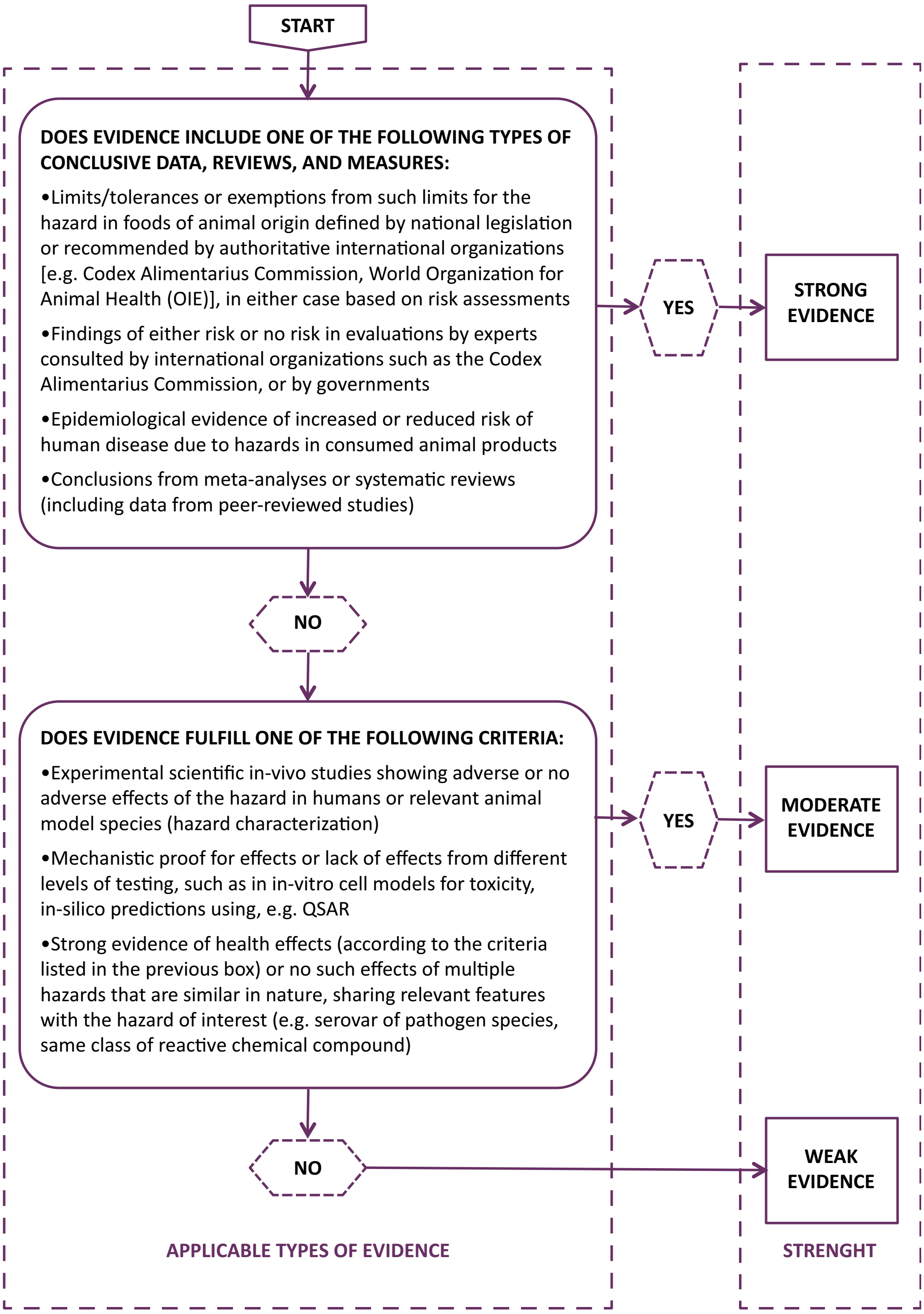
AT LEAST ONE OF THESE CONDITIONS APPLIES:

- Scientific data from systematic reviews, meta-analyses, or evidence of equivalent weight indicate that a significant transfer of the agent from feed to food of animal origin occurs
- The transfer of an agent from feed to food of animal origin has served as basis for regulatory limits to its levels in feed so as to prevent exceedance of safety thresholds in animal products for human consumption, which have been based on safety assessments by experts advising authoritative international organizations or multi-state governments or state associations, hence reflecting broad consensus and vast data availability

AT LEAST ONE OF THESE CONDITIONS APPLIES:

- Scientific data from systematic reviews, meta-analyses, or evidence of equivalent weight indicate that limited transfer of the agent from feed to foods occurs and that it is highly unlikely that limits for its presence in edible animal products will be exceeded
- Evaluations of a particular agent (e.g. chemical, microbial, physical) by experts advising authoritative international organizations, federal nations or associations of states have concluded that the transfer from feed to food products only has a limited role as a route of consumer exposure to the agent

Strength of evidence for potential impact on human health



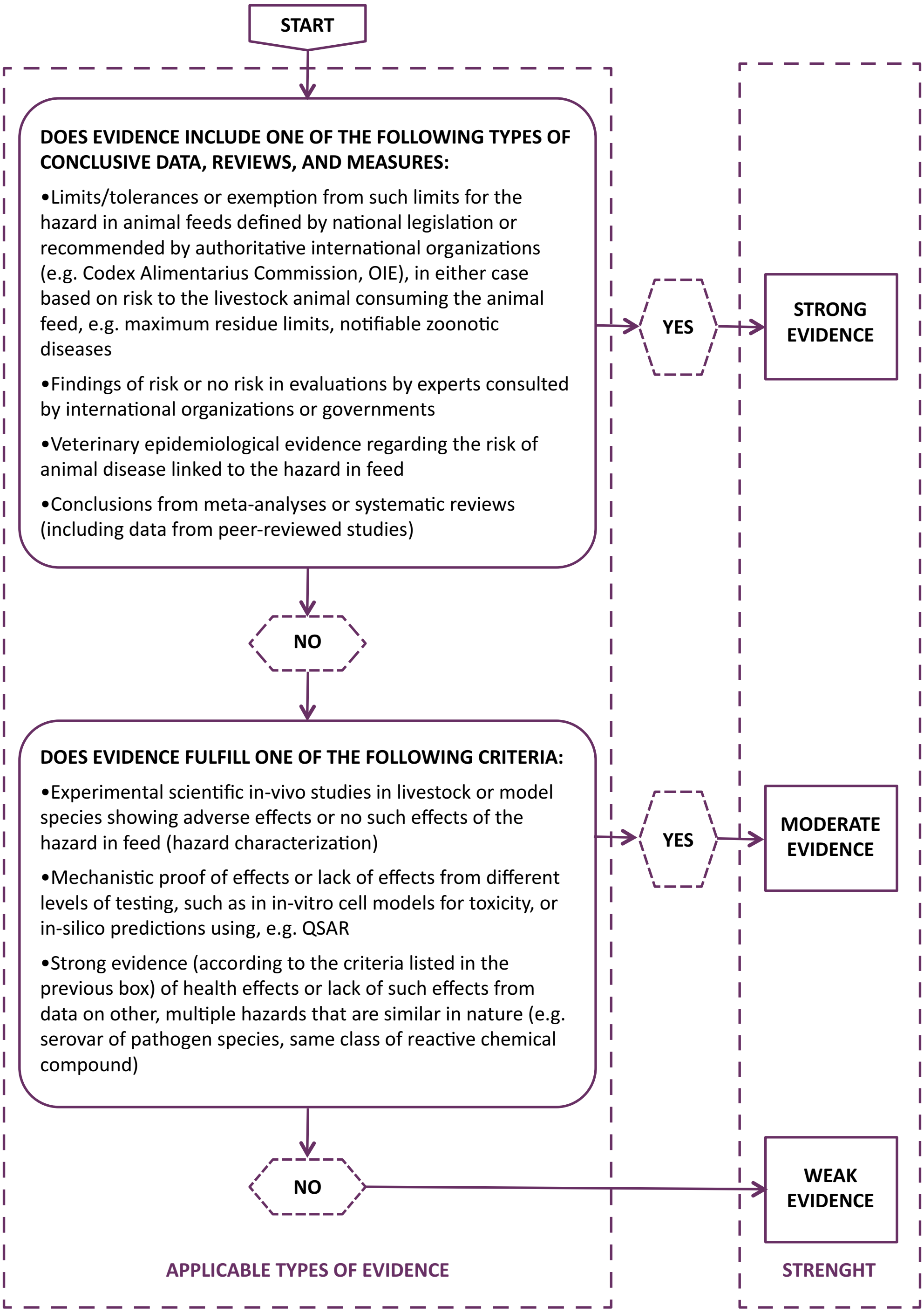
DOES EVIDENCE INCLUDE ONE OF THE FOLLOWING TYPES OF CONCLUSIVE DATA, REVIEWS, AND MEASURES:

- Limits/tolerances or exemptions from such limits for the hazard in foods of animal origin defined by national legislation or recommended by authoritative international organizations [e.g. Codex Alimentarius Commission, World Organization for Animal Health (OIE)], in either case based on risk assessments
- Findings of either risk or no risk in evaluations by experts consulted by international organizations such as the Codex Alimentarius Commission, or by governments
- Epidemiological evidence of increased or reduced risk of human disease due to hazards in consumed animal products
- Conclusions from meta-analyses or systematic reviews (including data from peer-reviewed studies)

DOES EVIDENCE FULFILL ONE OF THE FOLLOWING CRITERIA:

- Experimental scientific in-vivo studies showing adverse or no adverse effects of the hazard in humans or relevant animal model species (hazard characterization)
- Mechanistic proof for effects or lack of effects from different levels of testing, such as in in-vitro cell models for toxicity, in-silico predictions using, e.g. QSAR
- Strong evidence of health effects (according to the criteria listed in the previous box) or no such effects of multiple hazards that are similar in nature, sharing relevant features with the hazard of interest (e.g. serovar of pathogen species, same class of reactive chemical compound)

Strength of evidence for potential impact on animal health



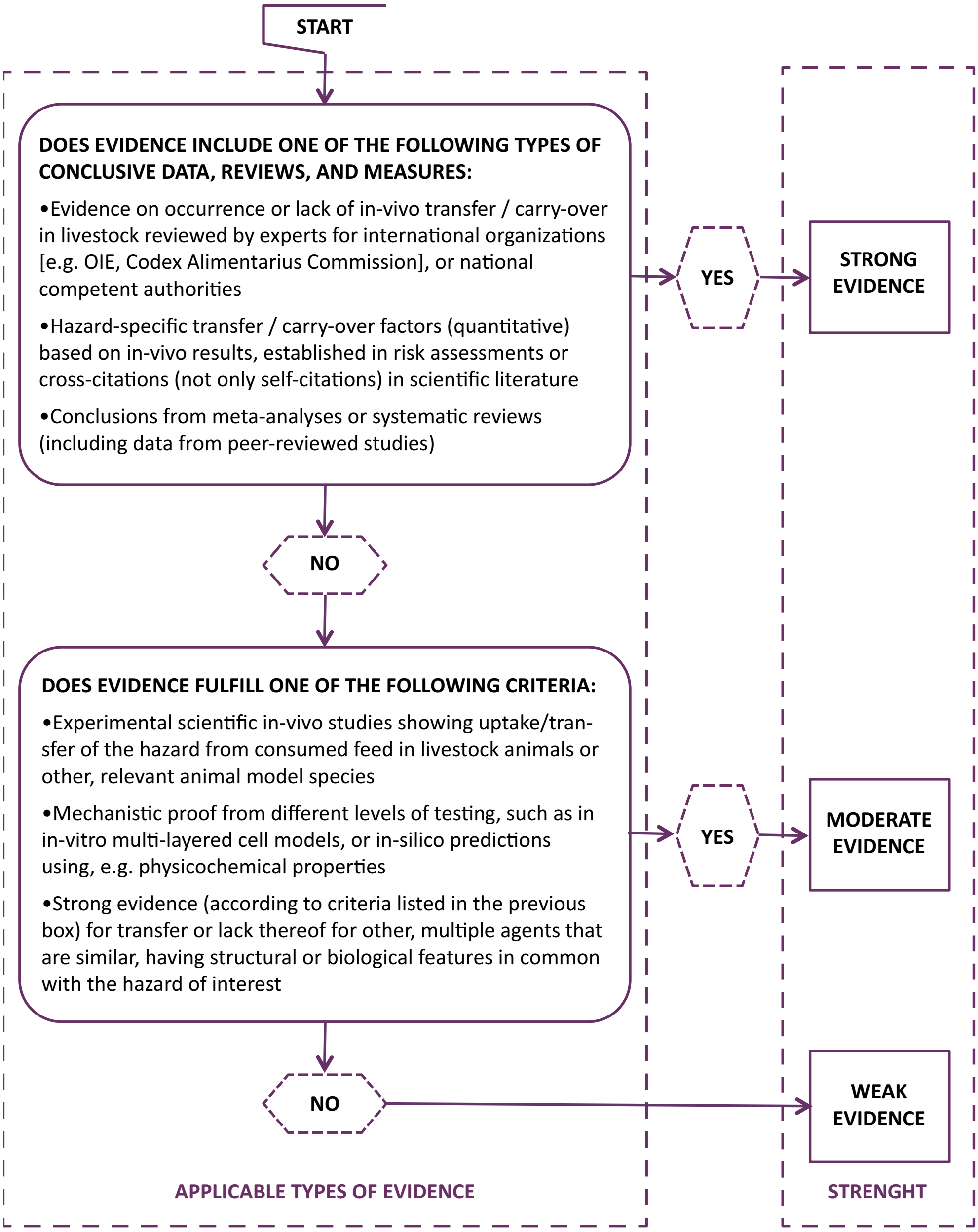
DOES EVIDENCE INCLUDE ONE OF THE FOLLOWING TYPES OF CONCLUSIVE DATA, REVIEWS, AND MEASURES:

- Limits/tolerances or exemption from such limits for the hazard in animal feeds defined by national legislation or recommended by authoritative international organizations (e.g. Codex Alimentarius Commission, OIE), in either case based on risk to the livestock animal consuming the animal feed, e.g. maximum residue limits, notifiable zoonotic diseases
- Findings of risk or no risk in evaluations by experts consulted by international organizations or governments
- Veterinary epidemiological evidence regarding the risk of animal disease linked to the hazard in feed
- Conclusions from meta-analyses or systematic reviews (including data from peer-reviewed studies)

DOES EVIDENCE FULFILL ONE OF THE FOLLOWING CRITERIA:

- Experimental scientific in-vivo studies in livestock or model species showing adverse effects or no such effects of the hazard in feed (hazard characterization)
- Mechanistic proof of effects or lack of effects from different levels of testing, such as in in-vitro cell models for toxicity, or in-silico predictions using, e.g. QSAR
- Strong evidence (according to the criteria listed in the previous box) of health effects or lack of such effects from data on other, multiple hazards that are similar in nature (e.g. serovar of pathogen species, same class of reactive chemical compound)

Strength of evidence for potential transfer to food of animal origin



DOES EVIDENCE INCLUDE ONE OF THE FOLLOWING TYPES OF CONCLUSIVE DATA, REVIEWS, AND MEASURES:

- Evidence on occurrence or lack of in-vivo transfer / carry-over in livestock reviewed by experts for international organizations [e.g. OIE, Codex Alimentarius Commission], or national competent authorities
- Hazard-specific transfer / carry-over factors (quantitative) based on in-vivo results, established in risk assessments or cross-citations (not only self-citations) in scientific literature
- Conclusions from meta-analyses or systematic reviews (including data from peer-reviewed studies)

DOES EVIDENCE FULFILL ONE OF THE FOLLOWING CRITERIA:

- Experimental scientific in-vivo studies showing uptake/transfer of the hazard from consumed feed in livestock animals or other, relevant animal model species
- Mechanistic proof from different levels of testing, such as in in-vitro multi-layered cell models, or in-silico predictions using, e.g. physicochemical properties
- Strong evidence (according to criteria listed in the previous box) for transfer or lack thereof for other, multiple agents that are similar, having structural or biological features in common with the hazard of interest

New insights in potential hazards of conventional feed and feed ingredients

In this chapter new insights in potential hazards of conventional feed and feed ingredients are reviewed. A subdivision is made based on chemical, biological and physical hazards. Most of the classes of hazards were also included in the report of the FAO/WHO Expert Meeting on Animal Feed Impact on Food Safety held in 2007 (FAO, WHO, 2008b). Some new, emerging hazards have been added.

CHEMICAL HAZARDS

The various chemical contaminants may actually occur as mixtures in feed and food. For some compounds, in particular chlorinated dioxins and dioxin-like PCBs, so-called toxic equivalency factors (TEFs) have been established based on studies that revealed differences in potencies of the individual compounds. For these compounds, the assumption is that they have similar toxic effects and mode of action (via the Ah-receptor), and are persistent. Other compounds share these properties like the brominated and mixed chloro-bromo analogues and certain polychlorinated naphthalenes (PCNs). However, these contaminants have not been assigned TEFs and as such been included in the maximum levels for food and feed. More information is required to study their levels and relative contribution to the overall toxic equivalency (TEQ) level. Also other groups of contaminants might be assigned TEFs based on other toxic effects and mode of action, like certain non-dioxin-like PCBs and PBDEs. More information is required to allow this approach. Recently EFSA proposed relative potency factors for estrogenic potencies of the various metabolites of zearalenone (ZEN). In particular zeranol or α-zearalanol (alfa-ZAL) has a much higher estrogenic potency than ZEN and can be formed in certain animal species. In the case of another mycotoxin, deoxynivalenol (DON), it was assumed that its glycoside and the two acetyl metabolites have similar toxic potency.

Compounds may also interact in different ways (potentiation, antagonistic), but more information is needed on how to deal with that in case of mixtures.

Contaminants

Dioxins and dioxin-like pcbs

Description of the hazard

Dioxins is a generic term used for polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs). In practice only the seven PCDDs and 10 PCDFs with at least four chlorines and containing chlorines at all four positions 2,3,7 and 8 are relevant since these tend to accumulate in the food-chain and the human body. Twelve PCBs, containing at least four chlorines and none (non-ortho) or just one (mono-ortho) chlorine at the ortho-position, have properties that are very similar to the more persistent PCDD/Fs. These PCBs are termed dioxin-like PCBs (dl-PCBs). For consumers, food of animal origin, including fish, is the most important source for exposure and since these compounds accumulate in fat, the more fatty products contribute most.

The different potencies of dioxins and dl-PCBs are reflected in so-called TEFs (Toxic Equivalency Factors), established and evaluated by an international expert working group, based on comparative studies. The last time this took place was in 2005, organized by WHO. The TEFs for PCDD/Fs vary between 1 for the most toxic congener (2,3,7,8-tetrachlorodibenzo-p-dioxin or TCDD) and 0.0003 for the octachlorinated dioxins, OCDD and OCDF. For dl-PCBs, the TEFs vary between 0.1 (PCB 126) and 0.00003 (for the 8 mono-ortho-PCBs) (Van den Berg *et al.*, 2006).

The combined levels of dioxins and dl-PCBs are expressed in so-called Toxic Equivalents (TEQs), following the correction of the absolute levels for the relative potencies of the various congeners, as expressed by the TEFs. TEQ-levels are typically in the lower ng/kg fat range in animal derived products and ng/kg product in feed (EFSA, 2010, 2012). In practice the dl-PCBs often contribute equally to the current background levels as PCDD/Fs, when expressed as TEQs. Patterns of the various congeners strongly differ for the various sources and may be an indication for the potential source (Hoogenboom *et al.*, 2015).

Sources

Increased monitoring of feed and food has resulted in the discovery of various contaminations and the elucidation of new sources in the past two decades (for review of incidents see Malisch and Kotz, 2014; Hoogenboom *et al.*, 2015). Dioxins are formed during incineration of certain materials and as such may also be formed during drying processes using inadequate fuels. Several incidents have occurred, e.g. with bread meal dried with painted wood in Germany (Hoogenboom *et al.*, 2004) or with fuels containing PCBs in Ireland (Heres *et al.*, 2010; Tlustos *et al.*, 2012; Marnane, 2012; Hoogenboom *et al.*, 2015). An overview of incidents is given in Table 2.

Waste incineration but also certain fires may result in the contamination of grass and fodders in the surroundings. Dioxins are also formed during production of

Dioxins and dioxin-like PCBs

Hazard	Dioxins and dioxin-like PCBs	
Source(s)	Anthropogenic and natural; elevated environmental levels in plants and soil in industrial areas; fishmeal and fish oil produced using fish harvested from contaminated areas; clay minerals; direct drying of feed materials, using inappropriate fuel.	
Transfer to food of animal origin	**Transfer:** high for milk and eggs; high for fish; medium for meat of farm animals; high for livers	**Strength of evidence:** strong
Potential impact on human health	**Potential impact:** high	**Strength of evidence:** strong
Potential impact on animal health	**Potential impact:** medium for poultry; low for ruminants, pigs and fish	**Strength of evidence:** moderate for poultry and fish; weak for ruminants and pigs
Knowledge gaps	• Limited information is available regarding the toxicity for farm animals, especially for ruminants and pigs • Limited data are available regarding environmental sources (worldwide) • Mainly for many developing countries, information about the presence of dioxins and dioxin-like PCBs in feed, feed ingredients and feed additives is rather scarce	

certain chlorinated substances such as chlorophenols, 2,4,5-T and polychlorinated biphenyls (PCBs). The use of pentachlorophenol (PCP) for preservation of wood is an important source which may occasionally cause problems in older stables (Ryan *et al.*, 1985; Feil *et al.*, 2000; Fries *et al.*, 2002), but also regarding the use of saw dust as carrier of vitamins (Llerena *et al.*, 2003) or wood chips as bedding material (Diletti *et al.*, 2005; Brambilla *et al.*, 2009). Although these materials are unlikely to pose a direct threat to animals, animal derived food products may become contaminated at levels exceeding maximum levels. Chlorophenols were also the source of dioxins in industrial fatty acids intended to be used for deinking but ending up in feed in Germany in 2010 (Abraham *et al.*, 2011; Hoogenboom *et al.*, in preparation).

Table 2: Incidents with PCDD/Fs and PCBs in the feed and food chain*

Country	Year	Source	Reference
US	1957	Feed fat, cow hides, chlorophenols	Schmittle *et al.*, 1958; Sanger *et al.*, 1958; Higgenbotham *et al.*, 1968; Firestone, 1973
US	1969	Water, chlorophenols	Firestone, 1973
Japan	1968	Rice oil; PCB-oil	Kuratsune *et al.*, 1972
Taiwan	1979	Rice oil; PCB-oil	Hsu *et al.*, 1985
Netherlands	1989	Waste incinerators	Liem *et al.*, 1991
US	1996	Ball clay, feed, chickens, cat fish	Cooper *et al.* 1995; Hayward *et al.*, 1999; Ferrario *et al.*, 2000
Germany	1997	Brazilian citrus pulp, lime, PVC	Malisch, 2000; Carvalhaes *et al.*, 2002; Malisch and Kotz, 2014
Belgium	1999	Feed fat, PCB-oil	Bernard *et al.*, 1999; Van Larebeke *et al.*, 2001; De Bont *et al.*, 2003; Traag *et al.*, 2006
Austria	1999	Kaolinic clay	Jobst and Aldag, 2000
Germany, Spain	2000	Choline chloride, sawdust, PCP	Llerena *et al.*, 2003
Italy	2001-2004	Mozzarella, waste incineration	Diletti *et al.*, 2008
Germany	2003	Dried bakery waste, waste wood	Hoogenboom *et al.*, 2004
Italy	2004	Wood shavings, PCP	Diletti *et al.*, 2005; Brambilla *et al.*, 2009
Netherlands	2004	Potato peels, kaolinic clay	Hoogenboom *et al.*, 2010
Netherlands	2006	Feed fat, gelatine, HCl	Hoogenboom *et al.*, 2007
Switzerland	2007	Guar gum	Wahl *et al.*, 2008
Chile	2008	Feed, zinc oxide	Kim *et al.*, 2011
Ireland	2008	Dried bakery waste, PCBs in fuel	Heres *et al.*, 2010; Tlustos *et al.*, 2012; Marnane, 2012
Netherlands, Germany	2010	Organic corn, unknown	RASFF 2010.0519
Germany	2010	Industrial fatty acids, chlorophenols	Abraham *et al.*, 2011; Hoogenboom *et al.*, in preparation
Germany	2011	Beet pulp, plastics in fuel	Hoogenboom et al, 2015
Various countries	2006-2014	(Hydrogenated) palm fatty acids distillate ((H)PFAD)	RASFF, Taverne-Veldhuizen *et al.* in preparation

* the issue with eggs from free-range hens is a more generic problem, with environmental sources like local waste burning and PCB-containing building materials. Environmental issues may occasionally also be important with other animal species foraging outside, e.g. in areas contaminated by municipal waste incinerators or fires, and flood plains of polluted rivers (cows, sheep).

Certain clay materials have also been shown to contain dioxins, probably formed under high pressure and temperature. The use of some of these contaminated clay materials, like kaolinic clay and Mississippi ball clay as feed additives caused contamination of animal derived products (Cooper *et al.*, 1995; Hayward *et al.*, 1999; Ferrario *et al.*, 2000; Jobst and Aldag, 2000). Kaolinic clay was also the cause of another incident due to its use in the potato industry and the feeding of contaminated peels to dairy cows (Hoogenboom *et al.*, 2010). Dioxins have also been found in certain minerals used as feed ingredients (Ferrario *et al.*, 2003), in some cases obtained during recycling (zinc-oxide) (EU-RASFF, Kim *et al.*, 2011). A particular case was described for CuSO4 used in animal feed (Wang *et al.*, 2011). This material was contaminated due to the use of HCl that was a by-product from the chemical production of chlorinated compounds. Similar might have been the case for the HCl used for the production of gelatine, resulting in the contamination of fat used for animal feed (Hoogenboom *et al.*, 2007). Recycled materials remain a threat for contamination of the food chain. It has been shown that also processing of fats and oils may be a critical step, due to e.g. concentrating of PCDD/Fs and dl-PCBs into certain fractions during distillation (various notifications in RASFF). It was also shown that dechlorination may occur during hydrogenation of PFADs into HP-FAD, resulting in the formation of lower chlorinated, often more toxic, congeners from octachlorinated congeners (Taverne-Veldhuizen *et al.*, in preparation).

Fishmeal and fish oil may contain relatively high levels of dioxins and dl-PCBs, and as a result also feed used in aquaculture (EFSA, 2012). A decrease in the levels is observed regarding the increased awareness of the aquaculture industry, the use of fish meal and fish oil sourced from areas of low contamination, decontamination procedures and the change from fish ingredients to materials of plant origin (Amlund *et al.*, 2012). The sum of dioxins (PCDDs and PCDFs) and dl-PCBs in commercial Norwegian fish feed decreased significantly from 2001 to 2010. None of the feed samples from any of the years had sum dioxin and dl-PCB levels above the EU maximum limit in feed. There was a significant correlation between the concentration of the sum of dioxins and dl-PCB and the inclusion of fish oil in fish feed (Sissener *et al.*, 2013). Monitoring results from the period 2001 to 2011 in feed materials applied in the Netherlands, covering in total 4938 samples, showed that the percentage of samples exceeding maximum levels for either dioxins or the sum of dioxins and dl-PCBs, set within the European Union, were below 1% for most feed categories, except for fish meal (4.1%), clay minerals (binders and anti-caking agents) (3.4%), and vegetable oils and by-products (1.7%) (Adamse *et al.*, 2015).

Dioxin-like PCBs are part of technical PCB-mixtures although their contribution to the absolute levels is relatively small. PCBs have been produced in the past and were used in e.g. transformers, but also as heat transfer fluid in equipment used in the food chain and in paints and sealants. Although the first two sources were actively removed in many countries, the latter sources are more disperse and as such more difficult to deal with.

It should be mentioned that in addition to feed, the environment and in particular soil may be an important source for these contaminants, in particular for animals foraging outside. This is partly due to the consumption of soil attached to grass but also the deliberate consumption of soil and small stones by chickens (Abrahams and Steigmajer, 2003). It should be stressed that in many countries there is a general background level in the environment with occasional hotspots due to local industries, spills, contaminated building materials or waste burning. This may lead to occasional problems on farms with e.g. chickens foraging outdoors (Piskorska-Pliszczynska *et al.* 2014, Hoogenboom *et al.* 2014).

Transfer to food of animal origin

Dioxins and dl-PCBs accumulate in edible tissues and organs from food-producing animals and are transferred to milk and eggs (Hoogenboom, 2012). Transfer to milk and eggs can be around 50% of the ingested dose at steady state conditions, but differs for the various dioxin and PCB congeners, due to differences in absorption and metabolism in the animal (Hoogenboom *et al.*, 2006, 2015). Also the strong growth of young animals is an important factor in the levels at the time of slaughter. Some of the dioxins (e.g. TCDF and 1,2,3,7,8-PeCDF) and PCBs (PCB 77) are actively degraded by certain species. The higher chlorinated dioxins are relatively poorly absorbed and transferred to eggs and milk. Also there is a selective accumulation (sequestration) of certain congeners in the liver, causing relatively high levels in livers of foraging animals, like sheep and deer (EFSA, 2011), but potentially also other animal species raised outdoors. At higher exposure levels, as during incidents, the accumulation in liver may even be increased. This is due to increased formation of cytochrome P450 1A2 in the liver (induction), resulting on the one hand in increased degradation but also increased binding. Overall, a larger part of the dioxins and PCBs will be present in the liver. As a result of these differences in toxicokinetics, the congener patterns in animal derived products will differ from the ones in the feed.

The transfer from fish feed to salmon fillets showed retentions of PCDD/Fs congeners of 10–34% of the administered dose after prolonged feeding with contaminated feed (Berntssen *et al.*, 2011). This shows that salmon quite efficiently stores these compounds in the tissues.

Potential impact on human health

Dioxins and dl-PCBs are classed by the IARC as a human carcinogen (Group 1) but they are not genotoxic. High levels cause chloracne in humans. Dioxins and dl-PCBs cause a number of adverse effects in humans and animals including effects on the reproductive, cognitive and immune system. These effects partly point to a disturbance of hormonal levels in the organism (endocrine disruption). In general, all adverse effects of these compounds are thought to be mediated by the binding to a very abundant cytosolic receptor, called the aryl hydrocarbon (AhR) receptor. Activation of the AhR-pathway causes the induction of a number of genes, many of them encoding for enzymes involved in the biotransformation of endogenous (including hormones) and exogenous compounds. Based on a number of adverse effects and using a body burden approach, WHO in 2000 derived a so-called health based guidance value (HBGV), in this case a Tolerable Daily Intake (TDI), of 1-4 pg TEQ/kg BW/day (WHO, 2000). JECFA established a similar value but extended to a monthly intake (TMI: 70 pg TEQ/kg BW/month), thereby acknowledging that the chronic intake and accumulation in the body is most relevant for the general population (JECFA, 2002). The former EU Scientific Committee on Food (SCF) derived a tolerable weekly intake (TWI) of 14 pg TEQ/kg BW/week (SCF, 2001). All these HBGVs are based on adverse effects of the most toxic congener, TCDD, in laboratory animals. However, the HBGVs apply to the sum of dioxins and dl-PCBs, expressed as Toxic

Equivalents (TEQs). The US-EPA more recently set a so-called reference dose of 0.7 pg TEQ/kg BW/day based on more recent studies on effects in humans exposed to the most toxic dioxin, TCDD, during the Seveso incident (US-EPA, 2012).

Potential impact on animal health

The major adverse effects in animals are already described in the previous section. Chickens are very sensitive showing so-called chicken oedema disease, wasting syndrome and decreased hatching of eggs, effects that led to the discovery of some of the major feed incidents with dioxins (Higgenbotham *et al.*, 1968; Firestone, 1973, Bernard *et al.*, 1999). There is much less information on adverse effects in other food-producing animals.

In fish, dioxins and dl-PCBs cause developmental lesions through activation of AhR-mediated pathways (King-Heiden *et al.* 2012). Classic symptoms include oedemas, haemorrhages and craniofacial-, spinal and tail deformities (Tillitt *et al.*, 2017). Embryonic exposure to TCDD has been shown to cause cardiotoxicity and impact vascular- and skeletal development in fish (Hornung *et al.*, 1999; Spitsbergen *et al.*, 1991). Developmental effects of dioxins and dl-PCBs on embryos may subsequently influence integrative functions, such as swimming performance in fish (Tillitt *et al.* 2017).

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Non-dioxin-like PCBs

Description of the hazard

PCBs are man-made chemicals produced as mixtures with different chlorination grade called e.g. Aroclors, Kaneclors or Clophens. Due to their much higher levels and hence more simple detection, PCBs have been monitored longer than dioxins. This refers in particular to the more abundant congeners, formerly termed indicator PCBs (PCBs 28, 52, 101, 118, 138, 153, 180). PCB 118 is a mono-ortho PCB and actually considered a dioxin-like (dl)-PCB. In EU legislation, this PCB was therefore removed from the set and the remaining 6 PCBs are now termed non-dioxin-like or ndl- PCBs. The six PCBs make up only part of the total PCBs in the various mixtures applied in the past. For the widely used Aroclors 1254 and 1260 this is about 1/3 of the total. This figure actually changes in edible products of animal origin caused by selective metabolism (EFSA, 2005). Due to the widespread use and contamination of the environment, ndl-PCBs can be detected in feed at low µg/kg feed (EFSA, 2012).

Sources

PCBs were produced as technical mixtures and used in electrical transformers, heat exchange equipment (used also for heating oils and fats) but also in certain paints and coatings. Due to their persistence in the environment, the production of PCBs was stopped but for the same reason, they are still around and may enter the food chain.

One of the first incidents was caused by the use of PCB-containing coatings in silos for animal feed (Willett and Hess, 1975). In 1968, the leakage of PCB-oil into rice oil caused the death of large numbers of chickens, due to the use of a by-product of the purification process in animal feed (Kuratsune *et al.*, 1972). This incident, known as Yusho, also affected large numbers of people, using the rice oil for cooking. Another large incident with PCBs in feed was the presence of up to 200 litres of PCB-oil in 60-80 tonnes of fat used to prepare animal feed in 1999 in Belgium (Bernard *et al.*, 1999, Hoogenboom *et al.*, 2004). In addition there were some incidents where PCB-containing fuel was used for drying of feed materials, as in the case of the Irish

Non-dioxin-like PCBs

Hazard	Non-dioxin-like PCBs	
Source(s)	Anthropogenic; technical PCB-mixtures for electrical transformers, heat exchange equipment, etc.; paints and coatings; fishmeal and fish oil produced using fish harvested from contaminated areas; direct drying of feed materials, using inappropriate fuel; pieces of old paints and sealants; leaking of equipment.	
Transfer to food	**Transfer:** high for milk and eggs; high for fish; medium for meat and tissue of farm animals	**Strength of evidence:** strong
Potential impact on human health	**Potential impact:** unclear due to mixtures with PCDFs and dl-PCBs	**Strength of evidence:** weak
Potential impact on animal health	**Potential impact:** unclear due to mixtures with PCDFs and dl-PCBs	**Strength of evidence:** weak
Knowledge gaps	Effects of very pure PCB standards on animals, to discriminate from dioxin-like effects; this should include less traditional congeners	

incident in 2008 (Heres *et al.*, 2010; Tlustos *et al.*, 2012; Marnane, 2012) but possibly also the incident in 2010 in the Netherlands with contaminated corn (Hoogenboom *et al.*, 2015). Burning of the PCB-oil results in the formation of PCDFs.

In the Netherlands, investigations on farms with contaminated eggs from free-ranging chickens showed that also certain building materials can be contaminated with PCBs. Some of the contamination problems were caused by the reuse of building debris in the courtyard of farms for water management. In other cases, old asbestos roof plates contained a coating with PCBs which over the years contaminated the area surrounding the stable (Hoogenboom *et al.* 2013). Also the use of sewage sludge as fertiliser may contaminate the soil and plants.

As in the case of dioxins and dl-PCBs, fish meal and fish oil may contain relatively high levels of ndl-PCBs, and as a result also feed used in aquaculture (EFSA, 2012). A decrease in the levels is observed regarding the increased awareness of the aquaculture industry, the use of fish meal and fish oil sourced from areas of low contamination, application of decontamination procedures and the change from fish ingredients to materials of plant origin (Amlund *et al.*, 2012).

Transfer to food of animal origin

The ndl-PCBs can be detected in most foods of animal origin, including fish, at typical levels in the low µg/kg fat range (EFSA, 2012). The carry-over of ndl-PCBs has been studied in a number of food-producing animals (reviewed by Hoogenboom *et al.*, 2012). These compounds accumulate in the fat, and are transferred to lipid-rich products like milk and eggs. With continued exposure, levels in the latter products gradually increase but eventually reach a steady-state level. There are clear differences in the uptake, metabolism, accumulation and excretion of the different ndl-PCB congeners with PCBs 138, 153 and 180 being the ones that accumulate most. Since analysis has focussed on the indicator-PCBs, much less information is available on other congeners.

Potential impact on human health

EFSA evaluated the adverse effects of ndl-PCBs in 2005 (EFSA, 2005), but was unable to derive an HBGV for human exposure, since the most critical adverse effects were similar to those caused by dioxin-like compounds, and as such were likely to be caused by dl-PCBs and possibly PCDFs present in the mixtures and even standards used in animal studies. Even very low amounts of dioxin-like compounds can explain such effects and special attention is needed to exclude their contribution in toxicological studies. JECFA (2016) came to a similar conclusion and did also not derive an HBGV. Ndl-PCBs can bind to different receptors and induce a variety of enzymes involved in biotransformation, which can subsequently affect hormone homeostasis, as shown for thyroid and steroid hormones, corticosteroids and retinoids. This also applies for their hydroxy and methyl sulfone metabolites (JECFA, 2015). With some exceptions, most studies were performed with commercial mixtures or indicator-PCBs and more information is needed on other congeners occurring in food.

Potential impact on animal health

In practice animals are exposed to a mixture of ndl-PCBs and dl-PCBs, even containing PCDFs. This is also true for many well controlled animal studies (EFSA, 2005). During incidents it appears that the most significant effects are actually caused by the dioxin-like compounds. This does not mean that ndl-PCBs cannot have additional and differential adverse effects, but whether these are relevant in comparison to co-exposure to dl-PCBs and PCDFs remains to be determined. Also other congeners with different biological actions may occur.

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Organochlorine pesticides

Description of the hazard

Major representatives of the group of organochlorine pesticides (OCs) are DDT, lindane (γ-HCH), α- and β-HCH, aldrin, dieldrin, endrin, chlordane, heptachlor, toxaphene (camphechlor), hexachlorobenzene (HCB) and endosulfan. These substances have been used extensively in the past as insecticides. Since 2001, the UN-Stockholm Convention on Persistent Organic Pollutants started activities to ban or restrict the use of these OCs, because their persistence, bio-accumulation in fatty tissues and adverse effects on human health and the environment (www.pops.int). Endosulfan is one of the few organochlorine pesticides that is still in use in some countries although the UN Stockholm Convention has agreed in 2011 to add endosulfan to the United Nations' list of persistent organic pollutants to be eliminated worldwide (United Nations, 2011).

Sources

Efforts to reduce the exposure to OC-contaminated feed have been successful. As an example, in Sweden, HCB and p,p-DDE levels in adipose tissue from bovines and swine declined significantly (resp. 6–8% and 10–12% per year) in the period from 1991–2004 in almost all studied regions of Sweden, mirroring a declined contamination of the animal feed used in milk and meat production (Glynn *et al.*, 2009). However, as shown, among others, by European RASFF alerts, pertaining to feed materials and feeds that originate both from Europe as well as from other continents (Adamse *et al.*, 2014), OCs are still found occasionally at levels above the regulatory limits. DDT is also used to control the spread of malaria by mosquitoes. Through direct use in wetlands, residues of DDT will re-enter the food chain if fish or seaweed products are used in animal feed or aquafeed (Rose, 2012).

Organochlorine pesticides

Hazard	Organochlorine pesticides (OCs): Major representatives are DDT, lindane (γ-HCH), α- and β-HCH, aldrin, dieldrin, endrin, chlordane, heptachlor, toxaphene (camphechlor), hexachlorobenzene (HCB) and endosulfan. These substances have been used extensively in the past as insecticides. DDT is also used to control the spread of malaria.	
Source(s)	Anthropogenic; Environmental contamination; Fatty feed materials of animal origin, especially fish derived products such as fish oil; Improper disposal of chemical wastes; For endosulfan, vegetable oils are a main contributor to dietary exposure.	
Transfer to food of animal origin	**Transfer:** high for DDT, β-HCH, aldrin, dieldrin, endrin, heptachlor, HCB medium for lindane, α-HCH, chlordane low for endosulfan Variable for toxaphene	**Strength of evidence:** strong
Potential impact on human health	**Potential impact:** high; Important representatives have been classified as Group 1, 2A or 2B-carcinogens	**Strength of evidence:** strong
Potential impact on animal health	**Potential impact:** medium – high Some OCs (DDT, endosulfan) are highly toxic for fish	**Strength of evidence:** weak – strong, depending on the compound and the animal species
Knowledge gaps	There are relatively few data on toxicity of OCs in various production animal species (EFSA, 2005b; EFSA 2006a).	

Moreover, as was shown for β-HCH in Italy, the improper disposal of chemical wastes can create large "hotspots" of pasture contamination and lead to transfer to foods of animal origin (milk) by OCs that have not been used since decades in developed countries (Sala *et al.*, 2012).

Feed materials of animal origin, especially fish derived products, are in general more contaminated than feed materials of plant origin. In aquaculture compound feed is recognised as the major source of contaminants, such as OCs. Oil obtained from pelagic fish species are the main source of OCs in aquafeed and farmed oily fish such as Atlantic salmon (Amlund *et al.*, 2012). The most dominant OCs in Atlantic salmon aquafeeds are HCB, DDT and its degradation products, dieldrin, chlordane and toxaphene (Berntssen *et al.*, 2010).

A study in South China showed that trash fish (defined by FAO (2005) as "fish that have a low commercial value by virtue of their low quality, small size or low consumer preference. They are either used for human consumption (often processed or preserved) or used for livestock/fish, either directly or through reduction to fish meal/oil"), used as feed material in aquaculture, contained relatively high concentrations of DDXs (sum of o,p′ - and p,p′-DDT, -DDD, and -DDE and p,p′-DDMU), especially p,p′-DDT. The mean and maximum values of DDXs were 417 and 7040 ng/g resp. It was concluded that the habit of direct use of trash fish as fish feeds has induced the accumulation of DDXs in aquatic species (Ying *et al.*, 2009).

The steady increase in production volume in aquaculture of 8–10% a year and the demand for sustainable fish farming that relies less on marine fish ingredients such as fish oil and meal, have resulted in increasing use of alternative oils and proteins, viz. vegetable oils and plant proteins, in aquafeeds (Amlund *et al.*, 2012). Most plant ingredients have lower levels of OCs than marine ingredients, and use of plant ingredients lowers the load of OCs in aquafeeds, leading to a decrease in the levels in fish (Nacher-Mestre *et al.*, 2009; Berntssen *et al.*, 2010).

Incorporation of novel protein and oil sources as feed ingredients potentially exposes farmed fish to contaminants that may otherwise be of limited significance, viz. endosulfan (Amlund, 2012). In a study with Nile tilapia from Brazilian fish farms, the highest pesticide values in feed samples were for endosulfan, which suggests a possible contamination from ingredients used in the feed manufacturing process, especially soybean oil (Botaro et al, 2011). About 50 % of the tested lots of soybean oil and soybean fatty acids used in The Netherlands as feed materials in the period 2001-2011 contained measurable amounts of endosulfan in the range of 0.001 – 0.71 mg/kg (Adamse *et al.*, 2014).

Transfer to food of animal origin

Depending on their physico-chemical characteristics, some substances are metabolized into naturally occurring and generally harmless constituents. Other substances are persistent and remain in the animal and in animal products intended for human consumption such as milk and eggs, see Table 4 (Kan and Meijer, 2007). These authors divided the OCs into three major classes, based on their accumulation in milk, eggs and fatty tissues: (1) low accumulation: compounds rapidly metabolized and excreted; (2) detectable accumulation; (3) high accumulation.

In farmed fish the retention of most OCs is in the range of 15 – 35 % (Amlund *et al.*, 2012). For HCB, toxaphene and DDT in Atlantic salmon retention was between

Table 4: Accumulation of OCs in milk, eggs and fatty tissues (Kan and Meijer, 2007)

Compounds	Accumulation
Aldrin and dieldrin	high
Chlordane	detectable
DDT	high
Endosulfan	low
Endrin	high
α-HCH	detectable
β-HCH	high
γ-HCH (lindane)	detectable
Heptachlor	high
Hexachlorobenzene (HCB)	high
Methoxychlor	low
Toxaphene (camphechlor)	variable

34 and 58 % (Berntssen *et al.*, 2011). Endosulfan is less persistent, biological breakdown plays a major role in the low transfer to fish (Botaro *et al.*, 2011; Amlund *et al.*, 2012).

Potential impact on human health

The dominant toxic effects of OCs are in the nervous system and the liver. Some OCs, e.g. DDT, also affect hormonal tissues, reproduction, foetal development and the immune system. OCs can also cause liver hyperplasia and/or liver tumours in experimental animals (EFSA, 2005b; EFSA, 2006a). Neurotoxic effects of endosulfan in both humans and animals are well documented. Exposure can induce a number of effects including liver and kidney toxicity, haematological effects, alterations in the immune system, and alterations in the reproductive organs (EFSA, 2006b).

Several OCs have been evaluated by the International Agency for Research on Cancer (IARC): Lindane is classified as carcinogenic to humans (Group 1) (IARC, 2015); DDT as probably carcinogenic to humans (Group 2A) (IARC, 2015); HCB, toxaphene, α- and β-HCH as possibly carcinogenic to humans (Group 2B) (IARC, 1987; IARC, 2001).

Potential impact on animal health

The dominant toxic effects of OCs are in the nervous system and the liver. Except for experimental animals there are relatively few data on toxicity in other animal species (see EFSA opinions, e.g. EFSA, 2005b; EFSA 2006a). For HCHs, neurotoxicity and liver effects have been reported in fish and ruminants (EFSA, 2005a). DDT is highly toxic to fish. In oral studies a no effect level of 6.25 mg/kg diet was derived for salmon (EFSA, 2006a). Endosulfan is toxic for some aquatic species, in particular fish. Toxicity was mainly studied in Atlantic salmon, where minor adverse effects in the intestine were observed following exposure to ≥ 5 µg /kg feed. In Nile tilapia, oral exposure showed effects on thyroxin level and thyroid hormone metabolism at a dietary concentration of 100 µg/kg (EFSA, 2006b; EFSA, 2011). Effects of endosulfan in animals have already been described under the previous heading.

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Emerging contaminants

Brominated flame retardants

Description of the hazard

In order to lower the chance of a fire and to slow down the development of a fire, various groups of compounds have been used. Many of these contain bromine and hence are called brominated flame retardants. The best know classes are the polybrominated biphenyls (PBBs), polybrominated diphenyl ethers (PBDEs) (EFSA, 2011a), hexabromocyclododecane (HBCDD) (EFSA, 2011b), tribromophenols (TBP) (EFSA, 2012a) and tetrabromobisphenol A (TBBPA) (EFSA, 2011c). However, there are also less well-known BFRs that were also recently reviewed by EFSA (EFSA, 2012b). Many of these compounds are relatively persistent and as such the production of a number of BFRs has been stopped.

Sources

There was one major incident related to BFRs in feed, being the Michigan case in 1973, where a mix-up occurred between Nutrimaster and Firemaster (a technical mixture with PBBs) (Carter, 1976, Fries 1985).

Feed additives may also be contaminated with BFRs. Batches of choline chloride were shown to be contaminated with BFRs (PBDEs, octabromo-1,3,3-trimethyl-1-phenylindane (OBIND), TBP) and moreover, as a by-product of the BFRs, also contained brominated dioxins (Traag *et al.* 2009). The cause of the contamination is unknown.

In general, information on the presence of BFRs in feed and feed materials is still limited, and as a result also the relative contribution of feed to the levels observed in food of animal origin. Since, as a result of environmental contamination, fish from certain regions has been shown to contain relatively high levels of certain BFRs (EFSA, 2011), also fish meal and fish oil used as feed ingredients may be an important source of these contaminants (Suominen *et al.*, 2011).

Transfer to food of animal origin

Very few studies have been performed to study the transfer of BFRs from feed to food of animal origin (Hoogenboom, 2012). Studies with PBBs and PBDEs showed that these compounds are readily excreted into the milk of dairy cows, with clear

Brominated flame retardants

Hazard	Brominated flame retardants	
Source(s)	Anthropogenic; technical BFR-mixtures; fish meal and fish oil produced using fish harvested from contaminated areas;	
Transfer to food	**Transfer:** high for milk and eggs; high for fish; medium for meat of farm animals;	**Strength of evidence:** low
Potential impact on human health	**Potential impact:** margin of exposure seems relatively large with some uncertainties on BDE-99	**Strength of evidence:** medium
Potential impact on animal health	**Potential impact:** margin of exposure seems relatively large	**Strength of evidence:** low
Knowledge gaps	Monitoring data for major feed ingredients; transfer from feed to food of animal origin; toxicity data on farm animals	

differences between the different congeners. Studies with laying hens also showed the high transfer of PBBs and PBDEs to eggs.

Dietary accumulation of PBDEs has been investigated in feeding trials with different fish species (Atlantic salmon, trout, carp, etc.): a wide range of congener-dependent accumulation was reported, ranging from less than 0.02 to 5.2 % for BDE 209 to more than 90 % for BDE 47 (EFSA, 2005).

Potential impact on human health

To study the potential effects, various PBDEs have been tested on rats and mice and the most sensitive effects were observed on the neurobehaviour of mice (EFSA, 2011a). Benchmark dose modelling resulted in BMDL10 values of 309, 12, 83 and 1,700 µg/kg bw for respectively BDEs 47, 99, 153 and 209, based on a single administration. For HBCDDs, the same endpoint in mice was used as the critical effect with a BMDL10 of 790 µg/kg bw, again based on a single administration. In addition these compounds, like dioxins and PCBs, have effects on the thyroid, possibly due to altered metabolism of hormones. For TBBPA, EFSA derived a BMDL10 value of 16.000 µg/kg bw for changes in plasma levels of thyroid hormones in female rats treated for 28 days. For PBBs, EFSA identified a NOEL of 150 µg/kg bw/day for hepatic carcinogenic effects in rats, which were treated with a technical mixture.

In its risk assessments (see under Description of the hazard) and based on exposure estimates, EFSA could not exclude a potential risk for consumers for certain PBDEs (in particular BDE 99) and these might deserve the highest attention at the moment. This is also true for some novel BFRs, primarily because of the rather limited data on occurrence and toxicity.

Potential impact on animal health

There are no specific risk assessments for farm animals. This would be hampered by the lack of data on actual effects in farm animals and on feed levels. Nevertheless some effects were described for the Michigan incident and in addition, studies in laboratory animals mentioned above may indicate the critical levels in other species.

The mix-up in the Michigan case (PBBs) was actually discovered by effects in dairy cows, like a decreased body weight and milk yield, and a very typical effect on hoof growth (Fries, 1985). When treated with 67 mg/kg bw Firemaster PB-6 for 60 days, cows showed thymic involution and atrophy (Moorhead *et al.*, 1977).

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Perfluoroalkyl Substances (PFASs)

Description of the hazard

Perfluoroalkyl substances (PFASs) are a large group of fluorinated compounds that have and still are used for various applications, like water resistance of clothing materials, oil resistance of wrapping materials and production of non-stick cookware. The most well-known compounds and most studied are PFOS (perfluorooctanesulfonate) and PFOA (perfluorooctanoic acid or C8). Since it was shown that these compounds are rather persistent, production was ceased and alternative PFASs were used, to some extent with similar properties. Numerous studies have shown the presence of PFASs in human blood.

Sources

PFASs can end up in drinking water prepared from contaminated surface or ground water since these substances are difficult to remove during purification. Furthermore, they can be present in sediments. Two incidents occurred in Germany, in which a contaminated soil improver was used (Kowalczyk *et al.*, 2012). Since PFASs can be absorbed by crop plants, like potatoes, maize, wheat, ryegrass (Stahl *et al.*, 2009; Lechner and Knapp, 2011), this may also result in elevated levels in certain types of feed. Silage and barley were identified as sources of PFOS and perfluoroalkyl carboxylic acids (PFCAs) with 8-12 carbons for cows (Vestergren *et al.*, 2013). In general fish shows the highest levels and as a result, also fish derived feed ingredients may be contaminated (Suominen *et al.*, 2011). High levels have also been observed in livers of wild boars, but the source remains to be elucidated.

Transfer to food of animal origin

A number of studies showed that certain PFASs can accumulate in tissues of farm animals and be excreted in milk of cows and sheep (Kowalczyk *et al.*, 2012, 2013; Vestergren *et al.*, 2013). Cows that were exposed to PFOS and PFCAs with 8 to 12 carbons through their diet contained detectable concentrations of PFOS and C8-10 PFCAs in muscle tissue and milk. Concentrations in liver and blood of these cows

Perfluoroalkyl substances

Hazard	Perfluoroalkyl substances	
Source(s)	Industrially produced compounds applied in consumer products; Exposure directly from the environment (soil, water), crops grown on contaminated soil and animal derived feed ingredients (in particular fish)	
Transfer to food of animal origin	**Transfer:** Medium for milk, liver and kidney. Low for meat. Unknown for eggs. Certain PFASs transfer to milk and may accumulate in liver and kidney.	**Strength of evidence:** Moderate
Potential impact on human health	**Potential impact:** Medium	**Strength of evidence:** Strong
Potential impact on animal health	**Potential impact:** Unknown	**Strength of evidence:**Weak
Knowledge gaps	• Limited data on contamination of feed and feed ingredients, including grass and silage • Limited or lacking data on transfer to food of animal origin, in particular to eggs • Limited data on potential effects in farm animals	

were higher compared to the muscle tissue (Vestergren *et al.*, 2013). In another cow study, PFOS and perfluorohexane sulphonic acid (PFHxS) appeared in milk at relatively high levels, PFOA and perfluorobutane sulphonic acid (PFBS) did not. PFOS showed highest levels in liver, followed by kidney and meat. PFHxS also showed some accumulation in tissues. Plasma levels for PFOS were much higher than in milk. The half-life for PFOS both in tissues and milk was very long (Kowalczyk *et al.*, 2013). Similar observations were made for two sheep fed contaminated corn (Kowalczyk *et al.*, 2012). Numata *et al.* (2014) showed the accumulation of various PFASs in muscle and especially liver of pigs. Half-life in plasma was rather long, in particular for PFHxS and PFOS. Yeung *et al.* (2009) showed the accumulation of PFOS, perfluorodecanoic acid (PFDA) and to a lesser extent PFOA in plasma, liver and kidney of broilers. PFOA levels decreased much more rapidly when transferred to clean feed.

Potential impact on human health

Based on rodent studies, EFSA derived in 2008 TDIs for PFOS of 150 ng/kg bw/day based on effects on thyroid hormone and lipid levels, and for PFOA of 1500 ng/kg bw/day based on liver effects, for both compounds based on experimental animals. A more recent assessment by ATSDR (2015) resulted in so-called Minimum Risk Levels of 30 and 20 ng/kg bw/d for PFOS and PFOA respectively, based on the same studies but taking into account differences in kinetics between animals and humans. US-EPA (2016 a, b), again based on animal studies and taking into account differences in kinetics, derived Reference Doses of 20 and 20 ng/kg bw/day. Several epidemiological studies show that in humans these compounds are associated with increased serum cholesterol levels and decreased vaccination response in children.

Potential impact on animal health

Although some studies studied effects in farm animals (in particular chickens), this was not systematically reviewed.

Knowledge gaps

More data are needed on levels in feed and feed ingredients, including potential hot spots. More data are also needed for transfer to food of animal origin, in particular to eggs. Based on exposure of farm animals, more data should be collected on potential adverse effects.

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Other emerging contaminants

The following groups of contaminants have been qualified as emerging contaminants but only limited scientific information is available and so no further details regarding these contaminants are given in this background document:

- Polychlorinated napthalenes (PCNs) (Clarke and Smith, 2011)
- Polychlorinated alkanes (PCAs), often referred to as chlorinated paraffins (CPs) (Clarke and Smith, 2011)
- Mixed brominated/chlorinated dioxins and biphenyls (PXDDs/PXDFs/PXBs)
- Organotin compounds, especially tributyltin (EFSA, 2004; Suominen *et al.*, 2011)

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Natural contaminants

Mycotoxins

Description of the hazard

Mycotoxins are toxic secondary metabolites produced by fungi that readily colonize feed and food crops. One fungal species may produce various mycotoxins, and the

Mycotoxins

Hazard	**Mycotoxins** **Important mycotoxins are aflatoxins (AFL), ochratoxin A (OTA), Fusarium mycotoxins (deoxynivalenol (DON), zearalenone (ZEN), fumonisins (FUM), T-2/HT-2 toxins), ergot alkaloids, phomposins and sporidesmin**	
Source(s) (list is not exhaustive)	AFL: maize, groundnuts, sunflower products, copra; OTA: cereal grains, pulses, nuts; Fusarium mycotoxins: wheat, barley, oats, maize, maize gluten feed; ergot alkaloids: rye, sorghum, tall fescue; phompopsins: lupin; sporidesmin: rye grass	
Transfer to food of animal origin	**Transfer:** • Medium for AFB1 to milk (as AFM1) • Low for AFB1 to eggs, meat, etc. • High for OTA in blood/serum • Medium for OTA in kidney and liver • Low for OTA in other products • Low for Fusarium mycotoxins • Low for ergot alkaloids • Low for phomopsins • Low for sporidesmin	**Strength of evidence:** • Strong for AFB1 to milk • Strong for AFB1 to eggs / meat • Strong for OTA in blood/serum • Strong for OTA in kidney / liver • Strong for OTA in other products • Strong for Fusarium mycotoxins • Moderate for ergot alkaloids • Strong for phomopsins • Strong for sporidesmin
Potential impact on human health	**Potential impact:** • High for AFM1 in milk • Inconclusive for OTA in kidney / liver • Not applicable for other mycotoxins, except maybe α-zearalanol (zeranol) in milk	**Strength of evidence:** • Strong for AFB1 in milk • Weak for OTA in kidney/liver
Potential impact on animal health	**Potential impact:** • AFL: High for pigs; medium for ruminants and poultry. Variable for fish • OTA: High for pigs; medium for poultry; low for ruminants • DON: High for pigs; medium for poultry; low for ruminants • ZEN: Medium – High for pigs; Low for other animals • FUM: High for pigs and horses; Medium for poultry and ruminants • T-2/HT-2: High for pigs; medium for poultry; low for ruminants • Ergot alkaloids: High for pigs; medium for poultry and ruminants • Phomopsins: High, primarily in sheep • Sporidesmin: High (dairy cows)	**Strength of evidence:** • AFL: Strong • OTA: Moderate • DON: Strong • ZEN: Strong • FUM: Strong • T-2/HT-2: Strong • Ergot alkaloids: Strong for pigs and poultry; moderate for ruminants • Phomopsins: Strong • Sporidesmin: Strong
Knowledge gaps	Impact of co-occurrence of mycotoxins on animal health (Streit *et al.* 2012) Extent of exposure of animals to modified mycotoxins (Berthiller *et al.*, 2013) and its toxicological impact (EFSA, 2014). For this purpose, properly validated and sensitive routine analytical methods for modified mycotoxins are required (EFSA, 2014). Less well-known and emerging mycotoxins, e.g. nivalenol, enniatins, moniliformin, andrastin A, roquefortine C, beauvericin, citrinin, patulin, mycophenolic acid, lollines, peramines: extend of exposure of animals through feed, transfer to food and impact on animal and human health. Potential impact of aflatoxins on effectiveness of vaccines in poultry (Gabal and Azzam, 1998).	

same mycotoxin may be produced by several fungal species. There is a wide variety of mycotoxins that can occur in feed materials (Monge *et al.*, 2013; Storm *et al.*, 2014).

The variation in mycotoxin occurrence and their concentrations result from the presence and spreading of certain mycotoxin producing fungi in various regions and from the various environmental conditions, such as temperature and humidity (Battilani *et al.*, 2012; Parikka *et al.*, 2012). Mycotoxins in feed materials that are considered most important, due to their presence and toxic effects include aflatoxins, ochratoxin A (OTA), deoxynivalenol (DON), zearalenone (ZEN), fumonisins, T-2 / HT-2 toxins and ergot alkaloids. These mycotoxins contaminate several crops worldwide and represent a significant hazard to feed and food chains (Binder *et al.*, 2007). In addition, trading of feed and food raw ingredients (Keener et al, 2014) might increase the chances of blends of mycotoxins coming from different origins (Binder *et al.*, 2007).

Sources

Aflatoxins B1, B2, G1 and G2 are produced by various Aspergillus spp. From this group of four aflatoxins, aflatoxin B1 occurs most often. Aflatoxins are found regularly in commodities produced in tropical and subtropical regions, such as peanuts, and maize. Aflatoxin contamination is most common in African, Asian, and South American regions, but also occurs in the warmer areas of North America and Europe (Wu *et al.*, 2011; Stoev 2015; Perrone *et al.*, 2014).

Ochratoxin A (OTA) is produced by Penicillium and Aspergillus species in multiple crops. Affected commodities include cereal grains and their finished products, pulses and nuts (EFSA, 2006; Wu et al, 2014).

Fusarium mycotoxins commonly occur in small grain cereals, like wheat, barley and oats, as well as in maize (Miller 2008). The crop is infected pre-harvest and mycotoxins are produced mainly during the field period. The trichothecene mycotoxins, which include DON, ZEN, NIV, and T-2/HT-2 toxins, are found in multiple cereal grains. Fumonisins are found quite often in maize (Wu *et al.*, 2011) but may also contaminate sorghum, wheat, barley and oats (WHO, 2017; Cendoya *et al.*, 2014; Cirillo *et al.*, 2003). DON is the most regulated mycotoxin in feed materials worldwide (van Egmond *et al.*, 2007). DON can be found in very high concentrations in wheat, especially in years when Fusarium spp. is at epidemic levels in North America and Europe (Van der Fels-Klerx *et al.*, 2013). Maize (and derived products) grown in temperate climate zones can contain DON and ZEN, whereas maize from (sub)tropical areas is more often contaminated with fumonisins and aflatoxins, especially after drought stress and/or insect damage (Petterson 2012). The highest concentrations of ZEN were reported for wheat bran, maize and products thereof (e.g. maize flour, corn flakes). (EFSA, 2011a). Silage and forage are significant sources of ZEN (Reed and Moore 2009; Skladanka *et al.* 2011).

Ergot alkaloids are produced by Claviceps fungi that occur in various small grain cereals, in which they appear as sclerotia in the developing ears (Daenicke *et al.* 2008; Blaney *et al.* 2011). Ergot alkaloids occur in various feeds, at varying levels (Korn *et al.*, 2014; Zachariasova *et al.*, 2014), mainly in cereal grains and cereal by-products, and in particular rye, sorghum and millet and by-products derived from them (EFSA, 2012a). In some parts of the world, e.g. in the United States, ergot alkaloids are also produced by Neotyphodium fungi in grasses, such as tall fescue (Lolium arundinaceum) (EFSA, 2012a).

Mycotoxin contamination during cultivation of cropped feed materials depends on local weather conditions (van der Fels-Klerx & Booij, 2010). Therefore, the presence of fungi and mycotoxins originating from crop infection can never be fully eliminated. With the expected climate changes trends (of warming, occurrence of non-conventional rains, severe droughts, and unexpected flooding) in different areas around the world, the overall conditions for production of pre-harvest mycotoxins can be reached more frequently (Marroguín-Cardona *et al.* 2014). These changing global environmental conditions may increase mycotoxin presence in feed. Other developments, in addition to climate change, include the increasing demand for food of animal origin and thus feed materials, global trade, long-term distance transport, increased shipment size and long duration storage of large batches. Each of these developments will put pressure on the quality of animal feed (Makkar & Ankers, 2014).

Symbiotic relationships between a grass and a fungus occur worldwide (McCulley *et al.*, 2014). Well known is the relationship between the fungus Epichloe coenophila (formerly known as Neotyphodium coenophialum) and tall fescue (Lolium arundianaceum, formerly known as Festuca arundinacea or Schedonorus arundinaceus) resulting in abundant presence of the mycotoxins peramines, lolines and ergot alkaloids in the fodder (McCulley *et al.*, 2014; Zbib, 2014).

Formation of mycotoxins during ensiling of roughages: Main groups of roughages preserved for feed through ensiling include green crops from pasture grass or grass/clover mixtures and maize. The crop is often pre-dried and compressed, sometimes with the addition of formic acid, molasses or bacterial cultures, in order to promote quick acidification. It is then packed and stored under anaerobic conditions in silos, clamps or big plastic coated bales (Petterson 2012). When air is trapped in the crop when the crop is not tightly packed, if air leaks into the silo when opened or through the plastic of the bale, several fungi may invade the crop, grow, and may produce several mycotoxins (Driehuis *et al.*, 2008; Storm *et al.*, 2014). Farm-scale ensiling experiments in Italy indicated that aflatoxins could increase when silage is exposed to air during conservation or during feeding (Cavallarin *et al.*, 2011).

High concentrations of roquefortine C and mycophenolic acid (up to 45 and 25 mg/kg, respectively) were detected in visibly molded areas in surface layers of maize silage (Driehuis et al, 2008). From a farm management perspective, the type and distribution of mycotoxins within the silo and the formation of mycotoxins after opening of the silo are key points (Cheli *et al.*, 2013).

Fate of mycotoxins during storage and processing of cereals and pulses for compound feed: Small grain cereals and leguminous seeds grown for feed production can be contaminated with mycotoxins during the cultivation period. Proper storage must prevent growth of the fungal population (already present), and subsequent increase of mycotoxin contamination (Adegoke and Letuma. 2013). When small grain cereals are not sufficiently dry at harvest or not dried additionally after harvest, contamination by so-called storage fungi may occur, for example by Penicillium verrucosum. Under conducive temperature conditions this may lead to the production of OTA (Zachariasova *et al.*, 2014). Studies on the fate of mycotoxins in small grain cereals and maize during processing have shown that mycotoxins are concentrated in the bran and germ fractions (Lancova *et al.*, 2008). Industrial milling technology is a very complex process and presents several key processing steps that differently influence mycotoxin repartitioning in wheat milling fractions. Published data confirm that milling reduces mycotoxin concentration in fractions used for human consumption, but concentrates mycotoxins into fractions commonly used as animal feed (Cheli *et al.*, 2013). For instance, a study into the presence of mycotoxins in maize gluten feed revealed high levels of ZEN and DON (Petterson 2012).

The by-products from both the production of ethanol bio-fuel (distiller's dried grains with solubles, DDGS; see section 4.3) and beer/lager brewing may contain elevated concentrations of mycotoxins, since the mycotoxins may concentrate in the residual products. An enrichment of DON and ZEN from maize to DDGS of 3–3.5 times has been reported for ethanol industrial plants with different processing parameters (Zhang & Caupert 2012; Pinotti *et al.*, 2016).

Decontamination and detoxification

Physical methods can reduce mycotoxins and may warrant further investigation, including visual sorting (Mutiga *et al.*, 2014; Pearson *et al.*, 2004), blending and dehulling (Siwela *et al.*, 2005). Visual sorting of maize reduced fumonisin by 65% (Mutiga *et al.*, 2014).

The toxic effects of animal feed contaminated with aflatoxins can be decreased by ammoniation (Hoogenboom *et al.* 2001; Safamehr 2008). This treatment is not allowed in many countries. Another procedure that has shown promise is ozonation (Prudente and King, 2002). Binders can be added to animal feed to reduce availability of mycotoxins in the digestive tract. Mineral adsorbents such as mineral clays are often used to bind aflatoxins and other mycotoxins (Rizzi *et al.*, 2003; Di Gregorio *et al.*, 2014). Activated carbon (Devreese *et al.*, 2014), binders based on yeast (Faucet-Marquis *et al.*, 2014) and mannanoligosaccharides (Zaghini *et al.*, 2005) are also applied.

Exposure of animals

Animals may be exposed to mycotoxins through compound feed containing cereal grains and their by-products, oilseeds and their by-products, etc., through contaminated feed materials or through silage and forage. The following specific aspects need to be addressed.

Exposure of (dairy) animals in pasture lands and rangelands: Pasture can be a significant source of ZEN exposure (Reed and Moore 2009, Salvat *et al.* 2012; Skladanka *et al.* 2009; Golinski *et al.* 2005; Lauren *et al.* 1988). Facial eczema has been reported in grazing dairy cows in Australia and New Zealand (Dairy Australia, 2013). Animals ingest the mycotoxin sporidesmin produced by the fungus Pithomyces chartarum which lives mainly on ryegrasses. The fungus grows on moist, dead grass and is relatively widespread in dairy areas in Victoria but only grows under very specific conditions. Facial eczema only occurs when the climatic conditions are suitable for rapid proliferation and production of large numbers of toxic spores. Dairy cattle can suffer from liver damage. Production losses arise from animal deaths, weight losses or reduced weight gain, reduced milk yield and reproductive performance (Dairy Australia, 2013).

Animals grazing on stubble can be exposed to high levels of mycotoxins, e.g. sheep grazing on lupine stubble in Australia are exposed to phomopsins and can develop lupinosis with associated liver damage and – in the worst cases – death (Battilani *et al.*, 2011; de Nijs *et al.*, 2013). Since the mid-1990s, "sweet" (low alkaloid) lupin varieties have been developed that are also resistant to Diaporte toxicus (Phomopsis leptostromiformis), the fungus that produces the phomopsins (EFSA, 2012b). The use of phomopsin-resistant lupin cultivars has greatly reduced the risk in Western Australia (Allen, 2009). Now that these resistant varieties are the only ones being grown in Australia, combined with routine cleaning of lupin seeds to remove infected seeds and to meet a standard requirement of no more than 3% discoloured seed, the problem of lupinosis is no longer occurring either in animals grazing on lupin stubble or in animals given feed incorporating lupin seeds (John Edgar, personal information). The current situation in other lupin-producing regions of the world is less well documented (EFSA, 2012b).

Exposure through straw: Straw is typically used as bedding material in stables but sows and calves may consume significant amounts. Levels of Fusarium toxins in straw may be high (Nordkvist and Häggblom, 2014; Häggblom and Nordkvist, 2015).

Transfer of mycotoxins to food of animal origin

When aflatoxin B1 (AFB1) contaminated feed is consumed by milk producing animals, like dairy cows, goats and sheep, the mycotoxin can be metabolised in the animal's body, and is excreted as aflatoxin M1 (AFM1) in the milk (Fink-Gremmels, J., 2008a; Rao and Chopra, 2001; Wu F, 2015). Although less potent than aflatoxin B1, aflatoxin M1 is also a carcinogenic compound. Summarizing the results of 9 experimental studies, Petterson (2012) concluded that the transfer rates from AFB1 in feed to AFM1 in milk show variations from 0.32 - 6.2%. Among others, this considerable variation is due to the cows' production levels: high milk producing dairy cows, revealed the highest transfer rates, viz. 2.6% - 6.2% (Petterson, 2012).

Ochratoxin A is fat soluble and can be found in blood (Flores-Flores *et al.*, 2015). This toxin is mainly stored in kidney and liver (Battacone *et al.*, 2010; Dohnal *et al.*, 2011; EFSA, 2004a; Denli and Perez, 2010) and is transferred into edible animal tissue (like meat) but this transfer is generally low (Battacone *et al.*, 2010). Ochratoxin may also be transferred to eggs, especially when feed contamination reaches high levels (EFSA, 2004; Zahoor *et al.*, 2012). Transfer to milk has been demonstrated in rabbits and humans, but is minimal in ruminants, owing to metabolism of ochratoxin A by the rumen microflora (WHO, 2002).

Transfer of Fusarium mycotoxins in the animal's body to animal products like meat, eggs, liver and milk is very limited, as are the consequences for human health via this routes. An exception may be α-zearalanol (zeranol), a metabolite of ZEN, which has been detected in milk of dairy cows. This may be relevant because this metabolite has a much higher estrogenic potency than ZEN (EFSA, 2016).

Potential impact on human health

Adverse human health effects, as far as relevant, are summarized in Table 5.

AFM1 is most toxic with possible carcinogenic effects to humans (Group 2B), according to the International Agency for Research on Cancer (IARC, 1993). The information on possible adverse health effects of AFM1 on humans is scarce. The limited experimental animal studies carried out to determine toxicity and carcinogenicity of AFM1 seem to indicate that AFM1 has a hepatotoxic and a hepatocarcinogenic potential. The acute toxicity of AFM1 seems to be similar or slightly less than that of AFB1 but its carcinogenic potency is probably one or even two orders of magnitude lower than that of AFB1 (EFSA, 2004b).

Based on sufficient evidence for carcinogenicity in experimental animals through a mechanism not known to apply in humans, IARC classified OTA as a Group 2B possible human carcinogen (IARC, 1993). The most sensitive adverse effect in several mammalian species is nephrotoxicity, and this is likely also to be true in humans. Although

Table 5: Adverse human and animal health effects associated with mycotoxins in feed

	Human health effects due to transfer to edible animal products	Animal health effects	Reference
Aflatoxins	Liver cancer (hepatocellular carcinoma) Liver damage Acute aflatoxicosis Immune suppression Stunted growth in children[2]	Aflatoxicosis Liver damage Immune suppression Reduced weight gain and productivity Lower eggshell quality in poultry	Wu et al, 2011 Petterson, 2012 EFSA, 2004b IITA, 2015
Ochratoxin A	Inconclusive	Adverse renal effects; nephropathy	Wu et al, 2014 EFSA 2004a WHO, 2002
Deoxynivalenol	Not applicable[a]	Gastrointestinal disorders, including vomiting Immune suppression Feed refusal, erduced weight gain and productivity Pigs are the most sensitive species	Wu et al, 2011 EFSA, 2004b EFSA, 2013
Zearalenone	Possibly estrogenic effects due to zeranol	Estrogenic effects; pigs are the most sensitive species	EFSA, 2011a EFSA, 2016
Fumonisins	Not applicable[a]	Equine leukoencephalomalacia Porcine pulmonary oedema Liver damage Immune suppression Reduced weight gain and productivity	Wu et al, 2011 EFSA, 2005
T-2 / HT-2 toxins	Not applicable[a]	Immunological and haematological effects; pigs are the most sensitive species	EFSA, 2011b
Ergot alkaloids	Not applicable[a]	Ergotism Decreased feed intake and weight gain Growing pigs are very sensitive	Dänicke & Diers 2013 EFSA, 2012a
Phomopsins	Not applicable[a]	Lupinosis; primarily in sheep, but natural outbreaks have also been reported in cattle, goats, horses and pigs	EFSA, 2012b
Sporidesmin	Not applicable[a]	Facial eczema, liver damage	Dairy Australia, 2013

[2] The human health effects described in this cell are the potential effects of AFM1, based on limited information and partly based on the effects of AFB1

[a] significant transfer from feed to food of animal origin

an association between the intake of ochratoxin A and nephropathy in humans has been postulated, causality has not been established (WHO, 2002; EFSA, 2006).

Potential impact on animal health

Exposure of animals to mycotoxins will affect their performance, health and welfare. Short term exposure to high/medium concentrations of mycotoxins in feed will often produce acute and specific toxic effects in animals (Petterson, 2012). Long-term exposure to lower levels of mycotoxins, which are more common in feed, will mainly result in economic losses due to lower performance, chronic toxic effects (reproductive toxicity) or reduced resistance to bacterial infections as many mycotoxins act as immunosuppressants. Animals vary in their susceptibility to mycotoxins, according to the age and the species and the specific toxin involved (Pier *et al.*, 1980). Degradation in the rumen may play an important role in the actual exposure of the animals.

An overview of adverse effects on animal health is given in Table 5. AFB1 is most toxic with carcinogenic effects for animals (Wu *et al.*, 2011; EFSA, 2004c), although in general this effect will not be relevant in practice due to the limited livespan of farm animals. AFB1 is considered to be a human carcinogen, classified by IARC in group 1 (EFSA, 2004c). Pigs are highly susceptible; calves, turkey and sheep are moderately susceptible; chickens and cattle are relatively resistant. Fish vary from highly susceptible to resistant (IITA, 2015). It has been observed that aflatoxin contamination of feed may reduce effectiveness of vaccines in poultry (Gabal and Azzam, 1998).

Ochratoxin A (OTA) has been associated with adverse renal effects (Wu *et al.*, 2014). Pigs and poultry are particularly sensitive; ruminants are less sensitive due to degradation of ochratoxin A by the rumen microflora (EFSA, 2004a).

The trichothecene mycotoxins cause growth impairment and emesis in multiple species. Fumonisin causes species-specific adverse effects (see Table 5).

Toxic effects of ergot alkaloids include decreased feed intake and weight gain. Globally, toxicosis of livestock as a result of consuming ergot contaminated feed has been widely reported. Pigs are the most sensitive species while poultry appear to be able to tolerate higher levels of ergot alkaloids than other livestock (EFSA, 2012a; Dänicke and Diers 2013). Ergot alkaloids produced by Neotyphodium fungi in grasses, such as tall fescue (Lolium rundinaceum) may give rise to toxicity, especially for ruminants and horses (EFSA, 2012a).

This toxicity may be prevented by deliberate application of novel endophytes that do not produce ergots alkaloids (Bouton, 2009).

In general, pigs are considered the most susceptible animals to mycotoxin contamination (Petterson 2012). In particular, they will suffer from lower growth and productivity when exposed to DON and ZEN. Ruminants are considered less sensitive to mycotoxin exposure. Rumen microflora can degrade and inactivate mycotoxins. However, rumen detoxification capacity might be saturable and can vary with changes in diet, mycotoxin burden, duration of exposure, health status and production stage (Fink-Gremmels, J., 2008b).

Other animals and other mycotoxins are also relevant. Several diseases are caused by the mycotoxins in grass (e.g. peramines and lolines; see earlier), such as ryegrass staggers, fescue foot and summer slump syndrome, causing significant economic losses (Menna *et al.*, 2012; Stowe *et al.*, 2013; Zbib *et al.*, 2014 McCulley *et al.*, 2014). Lupinosis in sheep, after lupine consumption and facial eczema in grazing dairy cows caused by sporidesmin have already been described under the heading "Exposure".

Knowledge gaps

- Co-occurrence of mycotoxins is frequently observed. Multi-mycotoxin studies reported 75%-100% of the samples to contain more than one mycotoxin (Streit *et al.* 2012). Co-occurrence and statistical correlations between mycotoxins in feedstuffs collected in Asia-Oceania have been reported by Borutova *et al.* (2012). A survey on a very limited number of mycotoxins has already demonstrated that almost half of 7049 feed ingredients and compound feeds screened, were contaminated by more than two different mycotoxins out of the five investigated (Rodrigues and Naehrer, 2012). The toxicological effects of this co-occurrence are largely unknown. Synergistic effects between mycotoxins present in feed ingredients and diets could cause higher impact on animal health than the effects of the individual mycotoxins (Speijers and Speijers, 2004). Recently, JECFA evaluated toxicological data regarding co-exposure of aflatoxins and fumonisins (WHO, 2017). JECFA concluded that there are few data available to support co-exposure as a contributing factor in human disease. However, the interaction between AFB1 and fumonisins remains a concern. This is due to the fact that the incidence of chronic liver disease and stunting are high in the areas of the world where the exposures to both mycotoxins are high and the co-exposure has been confirmed with biomarkers (WHO, 2017). In vitro studies of mycotoxin combination toxicity showed antagonist, additive or synergic effects depending on the tested species, cell model or mixture, and were not necessarily time- or dose-dependent (Smith *et al.*, 2016; Wan *et al.*, 2013; Alassne-Kpembi et al, 2013; Oh *et al.*, 2012; Oh *et al.*, 2013).

- Modified mycotoxins: Plants can metabolize mycotoxins and store the modified mycotoxins in the vacuoles or cell walls. These modified (or conjugated) mycotoxins may be converted to their parent compound in the digestive tract of animals. The extent of exposure to the mycotoxins is unknown since only very few of these modified mycotoxins are detected during routine analysis, also due to the very limited availability of suitable standards (Berthiller *et al.*, 2013; EFSA, 2014).
- New mycotoxins: more insights in metabolism of fungi and plants as well as analytical developments currently lead to discovery of new (not formerly known) mycotoxins in feed. As an example, Storm *et al.* (2014) report the presence of, among many other mycotoxins, enniatin B and andrastin A in maize fodder.

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Plant toxins

Description of the hazard

Plant toxins are secondary metabolites produced by plants. The molecular structure varies from e.g. small simple calystegines to the complex dimer protein structure of ricin. The toxins can be produced by one family of plants but may as well be produced by several families (van Raamsdonk *et al.*, 2015). Typical examples are lectins, phytoestrogens, tropane alkaloids, pyrrolizidine alkaloids, quinolizidine (or lupin) alkaloids, cyanogenic glycosides, phorbol esters, colchicine, gossypol, furanocoumarins and alkenylbenzenes.

In the FAO/WHO expert meeting in 2007 (FAO and WHO, 2008), toxic plants were considered as "an undesirable substance of concern in feed". The experts defined toxic plants as plants having direct toxic effects on animal health, and having the potential for transfer of toxic compounds to milk and meat. They also identified lack of information about metabolic fates, residues, regulatory limits and health based guidance values (HBGVs) and concluded that the risk pathway can be controlled by following Good Agricultural Practices (FAO and WHO, 2008). Since then much work has been carried out on the various aspects of plant toxins but the complexity of the issue makes it difficult to design a general approach.

While some plants occur ubiquitously around the world, others are restricted to certain geographical areas, such as Indigofera spp. that occur in tropical and subtropical regions (FitzGerald *et al.*, 2011). Moreover, susceptibility between animal species can differ. This means that issues connected to plant toxins can be very local. Concentrations of plant toxins can vary in the season and between years (Pfister

Plant toxins

Hazard	Plant toxins	
Source(s)	Plants consumed by the animals while grazing, preserved roughages (e.g. hay), grain, compound feed, by-products from the bio-fuel production (e.g. rapeseed meal). Note: Plants and toxins of concern may be very different in various regions of the world.	
Transfer to food of animal origin	**Transfer:** some toxins with genotoxic and carcinogenic properties show transfer to meat, milk and eggs. This has been reviewed for pyrrolizidine alkaloids (PAs). The rate of transfer depends on the type of PA.	**Strength of evidence:** strong for PAs; weak for many other plant toxins
Potential impact on human health	**Potential impact:** low/medium	**Strength of evidence:** medium for PAs; weak for many other plant toxins
Potential impact on animal health	**Potential impact:** medium to high	**Strength of evidence:** medium
Knowledge gaps	• Limited data are available about the presence of plant toxins world-wide in pastures, rangelands and roughages and their potential hazards, taking into account regional differences. • Effects of climate change and changes in use of herbicides on the occurrence of types of toxic plants and concentrations of plant toxins. • Risk assessments are required to accurately characterize the types of hazards and the dose–effect relationships. • Limited data on transfer of many plant toxins.	

Legend: Impact can be high / medium / low; Strength of evidence can be strong / moderate / weak *et al.*, 2011, Cook *et al.*, 2015a). Overall, hundreds of plants and related toxins are reported to be potentially relevant for animal health.

Regional or local patterns in the use of feed products (e.g. roughages, food by-products) may change due to factors such as food and feed security, societal trends and economic factors. When alternative feed products are introduced, potential hazards related to plant toxins should be taken into account, e.g. for gossypol in cottonseed (Zhang *et al.*, 2007; Broderick *et al.*, 2013), ANFs in peas and faba beans (Gatta *et al.*, 2013) and ANFs in canola meal (Plaipetch and Yakupitiyage, 2013) and ANFs in rapeseed meal from biofuel production (Panda and Sastry, 2007).

Systematic reviews on the occurrence of specific toxic compounds in feed are rare. Some data on occurring plant toxins are reported for e.g. swainsonine and calystegine in Ipomoea carnea (Cook *et al.*, 2015a), anti-nutritional factors (ANFs) in flax seed flours (Russo and Reggiani, 2013) and the saponin protodioscin in several species of Bracchia, Panicum and Andropogon (Gracindo *et al.*, 2014). Mulder *et al.* (2009) reported on PAs in fodder in the Netherlands. On the other hand, many lists of poisonous plants, their main toxins and toxic effects to livestock in various regions of the world are available, e.g. for Australia (Meat and Livestock Australia), Saudi Arabia (Sharawy and Alshammari, 2009), USA and Canada (USDA, undated; Canadian Government, 2014; Cornell University, 2015; Mulligan, undated; University of California, undated), Spain, Portugal and Central America (Villar, and Ortiz Díaz, 2006) and the Netherlands (Wageningen University & Research - RIKILT, 2012; van Raamsdonk *et al.*, 2015). Data on certain plant toxins occurring in European feed can also be found in the EFSA opinions as mentioned in Table 6.

A survey regarding regulations is beyond the scope of this review. For plant toxins the situation seems to be quite diverse. For some of the plant toxins that have already been recognized since long as hazardous, maximum limits have been set in at least some parts of the world. This is e.g. the case for hydrocyanic acid, formed

Table 6: Opinions of EFSA on plant toxins in feed in Europe

Food and feed	
Erucic acid in feed and food	(EFSA, 2016)
Pyrrolizidine alkaloids in food and feed (2011)	(EFSA, 2011)
Tetrahydrocannabinol in milk	(EFSA, 2015a)
Tropane alkaloids in food and feed (2013)	(EFSA, 2013)
Feed – toxic compounds	
Cyanogenic compounds in feed (2007)	(EFSA, 2007)
Glucosinolates in feed (2008)	(EFSA, 2008a)
Gossypol in feed (2009)	(EFSA, 2009a)
Phorbol esters in Jatropha kernel meal (2015)	(EFSA, 2015c)
Ricin in feed (2011)	(EFSA, 2008b)
Saponins in Madhuca Longifolia L in feed (2009)	(EFSA, 2009b)
Theobromine in feed (2008)	(EFSA, 2008c)
Feed – plant material	
Ambrosia spp in feed (2010)	(EFSA, 2010)
Solanum glaucophyllum leaves in feed (2015)	(EFSA, 2015b)

from cyanogenic glycosides, to protect animal health. Also, for some toxic plants, levels have been set indirectly, e.g. based on seed counts in grain or maximum content of seeds / fruits / husks in feed ingredients and compound feeds (e.g. *Ricinus communis*). For emerging plant toxins (e.g. pyrrolizidine alkaloids (PAs)), as far as is known, no regulatory limits are set for feed or animal derived food.

Source

Main routes of exposure of animals to plant toxins are via plants consumed by the animals while grazing (van Raamsdonk *et al.*, 2015), when feeding preserved roughages contaminated with toxic plants (e.g. hay containing colchicine), grains, compound feed (van Raamsdonk *et al.*, 2009), by-products from bio-fuel production (e.g. rapeseed meal; Quiniou *et al.*, 2012) and, in very rare situations, via drinking water (Bonadies *et al.*, 2011).

Toxic plants in (semi-) grasslands/pastures and rangelands. Animals for dairy production (cattle, buffalo's, goats, sheep, llama's, camels) are kept relatively close to the farm, with regard to the milk collection and often graze on grasslands with a certain degree of management. This may include the removal of certain weeds, like ragwort, but not necessarily all types of weed. Consumption of certain weeds may be avoided by the animals due to palatability. However, their diet is often complemented with preserved roughage and compound feed. Furthermore, animals may be kept inside continuously or during the winter season and in that case their diet consists of preserved roughage and compound feed only. In that case animals can no longer recognize toxic weeds present in the feed. Cultivated grasslands pose the risk of weeds and their toxins, such as colchicine or PAs (Winter *et al.*, 2014; van Raamsdonk *et al.*, 2015). In Switzerland, the moment of cutting the pasture in the growing season was shown to be critical for eradicating Colchicum autumnale in grasslands that were under nature conservation regulations (Winter et al., 2014).

Animals for meat production can be kept on similar pastures, but in many parts of the world, they are often raised on more extensive natural pastures with a broader range of the regional flora (Pfister *et al.*, 2011; Chenchen *et al.*; 2014; Gracindo *et al.*, 2014). Again, these animals in general avoid the consumption of hazardous plants. However, climatic (no rain or abundant rainfall) (FitzGerald *et al.*, 2011; Fletcher *et al.*, 2011) or societal factors such as expansion of settlements (Abebe *et al.*, 2012) can force the animals to consume the high risk toxic plants.

Laying hens foraging outside tend to consume all vegetation in the outdoor area, including weeds present. However, the impact on their health is unclear.

Plants and plant toxins present in feed ingredients and compound feed. In Australia, grain crops may be contaminated with PAs, caused by the presence of weeds from *Heliotropium europaeum, Echium plantagineum, Symphytum* spp. and *Crotalia retusa*. The levels of PAs found in Australia were reported to range from<50 to >6000 µg/kg (Australia New Zealand Food Authority, 2001). PAs were also detected in various feed ingredients, including e.g. alfalfa, in the Netherlands. In the latter case common groundsel (*Senecio vulgaris*), a widely occurring weed, caused the contamination (Mulder *et al.*, 2009). Soy and other leguminoses such as lupine, are commonly used in compound feed for farm animals and fish worldwide (de Carvalho *et al.*, 2013). These feed materials have a history of safe use, but often after treatment to reduce the anti-nutritional factors (ANF) (Lim *et al.*; 2010, Gatta *et al.*, 2013). Several toxic plants that are regulated in the EU were detected in feed ingredients and compound feed, e.g. thorn apple (*Datura stramonium*), castor oil plant (*Ricinus communis*), and *Crotalaria* spp. (van Raamsdonk *et al.*, 2009). Rest materials of oil production, such as rapeseed and flaxseed meal, usually contain ANFs and must be processed before use (Panda and Sastry, 2007). The risks of the use of biofuel by-products from crops of increasing relevance are discussed in section 4.3. The variability in concentrations of plant toxins makes some varieties of flaxseed more suitable for use as broiler feed than others, as shown for cyanogenic glycosides, phytic acid, condensed tannins and trypsin inhibitors (Russo and Reggiani, 2013). In Africa, cassava leaves are used as feed ingredients. These leaves may also contain high levels of cyanogenic glycosides (Cocker, 2014).

Herbs and herbal extracts. Herbs or spices are applied for medicinal or health enhancing effects since ancient times. However, medical and veterinary offices, including National Toxicological Centres, have reported adverse effects of a range of different herbs or spices (Frohne and Pfänder, 2005).

The field of phytogenics describes the use of herbs as a special group of natural growth promoters (Brambilla and de Filippis, 2005; Máthé, 2009; Franz *et al.*, 2010; Steiner, 2006; Kostadinović and Lević, 2012; Alloui *et al.*, 2014), either as complementary feed (mainly horses), as feed additives or in premixtures (other farmed animals). Currently, the mainly used herbs and herbal extracts are those containing essential oils (thyme, oregano, mint, basil i.a.), thiosulphinates (garlic), coumarin (cinnamon) or saponins (yucca). Essential oils are the concentrated hydrophobic liquids in plants, that contain volatile aroma components. They are found in a diverse range of plants, but some plant families are particularly known for their high concentrations, primarily the *Lamiaceae, Apiaceae, Asteraceae* and *Rutaceae* (EFSA, 2012). The range of constituents in essential oils includes alcohols, aldehydes, esters, ethers, ketones, phenols, alkenylbenzenes, terpenes and furanocoumarins with as typical examples eugenol, carvacrol, eucalyptol, camphor, estragole and asarone (Benchaar *et al.*, 2008; Patra and Saxena, 2010; EFSA, 2012).

Effects of processing

Possible decontamination processes to reduce the ANFs or phytoestrogen activity of raw materials to be used in compound feed are heating and fermentation (Panda and Sastry, 2007; Zhang *et al.*, 2007; Jezierny *et al.*, 2010; Lim *et al.*, 2010; Broderick *et al.*, 2013; Gatta *et al.*, 2013; Plaipetch and Yakupitiyage, 2013). Lectins in soybeans are routinely inactivated by heating (toasting). Sometimes organic solvents are used to remove ANFs (Devanaboyina *et al.*, 2007). In addition, a decontamination process was developed for removal of cyanogenic glycosides from linseed cake (EFSA, 2017) and for phorbol esters from Jatropha cake (EFSA, 2015). Roughage can be dried or fermented when ensiled. This may reduce the amount of toxins, e.g. PAs (Crews *et al.*, 2009) or tannins from sorghum (Etuk *et al.*, 2012).

Transfer to food of animal origin

For most plant toxins the potential exposure of consumers via plant derived food will be much higher than via food derived from animals exposed via feed. Furthermore, high intake levels may result in adverse effects in the animals and as such prevent high levels in meat, milk and eggs, assuming that diseased or dead animals are not slaughtered and used for consumption. A possible exemption are compounds with genotoxic and carcinogenic properties, since chronic exposure of humans to even low levels may be relevant, whereas such effects are less likely to result in adverse effects in farm animals due to a relatively short life span. This applies e.g. to PAs. For PAs present in ragwort (Senecio jacobea) an overall transfer rate of 0.1% to milk in dairy cows was observed (Dickinson *et al.*, 1976, Hoogenboom *et al.*, 2011; Wang *et al.*, 2012a), although for individual congeners (e.g. jacoline, senkirkine), it was much higher. Therefore, the levels in milk largely depend on the intake but also the type of weed and inherent PA profile. The transfer of PAs to milk was also described for goats (Dickinson, 1980; Deinzer *et al.*, 1982). Studies with laying hens given different types of weed confirmed that similar is the case for the transfer to eggs (Edgar and Smith, 2000; Diaz *et al.*, 2014; Mulder *et al.*, 2016). In the latter study PAs were also detected in meat and liver of the animals, when slaughtered shortly after the last treatment. PAs were also reported in calves fed with Senecio brigalowensis, including protein adducts, for which the relevance for human health is unclear (Fletcher *et al.*, 2011).

Transfer of plant toxins to meat, e.g. indospicine (an amino acid, analogue of arginine, occurring in the free form only) (Tan *et al.*, 2014) is also known. Parthenium hysterophorus can taint meat, thus reducing the value of the meat products (Tudor *et al.*, 1982; Macconnachie *et al.*, 2010).

Potential impact on human health

Concerning potential effects on humans due to consumption of animal derived products, plant toxins with genotoxic and carcinogenic properties may be most relevant. This applies in particular to PAs. There are hundreds of different PAs and the composition varies for each plant species. There are no endemic regions reported where an increased cancer incidence was related to exposure to PAs in food. However, there were several incidents where exposure to PAs resulted in liver disease (Tandon *et al.*, 1976; Mattocks, 1986; Chen and Huo, 2010; Kakar *et al.*, 2010; EFSA, 2011). In the latter case in Afghanistan, flour was contaminated but also milk from goats. A number of PAs has been tested in chronic studies with rodents, resulting in liver tumours like hepatocellular carcinomas and haemangiosarcomas (EFSA, 2011). In particular the National Toxicology Programme (NTP) studies with lasiocarpine and riddelliine were used to derive a reference point for the risk assessment by e.g. EFSA. Since many PAs also showed genotoxic properties, PAs in general are regarded as genotoxic carcinogens for which no HBGV can be derived. Exposure levels of humans should be at least 10,000-fold lower than the BMDL10 values calculated from the rat studies. However, there are differences in the relative toxic potencies of PAs and recently Merz and Schrenk (2016) proposed relative potency factors (RPFs) for a number of the more common PAs. Thus far RPFs are not applied in routine analysis, where the levels of a selected number of PAs are summed into one overall level. Recently, EFSA (2017), like JECFA (WHO, 2016), decided to derive the BMDL10 from the rat study with riddelliine rather than lasiocarpine, as was done in a previous assessment (EFSA, 2011). This resulted in a BMDL10 value for haemangiosarcomas of 237 as compared to 70 µg/kg bw/day previously. EFSA also concluded that exposure via various types of tea and honey may result in MOEs smaller than 10,000. Other types of food, including animal derived products, contribute much less to the general exposure.

Potential impact on animal health

All farm animals (cattle, camels, horses, pigs, poultry, buffalo, hamsters, rabbits, sheep, and goats) and fish (salmon, trout) can be affected by plant toxins. Toxic effects in farm animals are reported worldwide, such as lantana poisoning in animals in tropical and subtropical regions (Sharma *et al.*, 2007), oak poisoning (Cortinovis and Caloni, 2013) and toxic effects after ingestion of ptaquiloside polluted water (Sharma *et al.*, 2013). The effects of the toxins on animal health are complex, varying from acute toxicity (Frohne and Pfänder, 2005) to more general effects (Blaney *et al.*, 2010; Etuk *et al.*, 2012; Fink-Gremmels, 2012). Several recent examples of acute toxicity are presented in Table 7. In most cases, the relationship between the consumption of specific toxic plants and the toxic effect has been established but in general, it is not always clear which specific plant toxins and which doses caused this toxic effect. Overall effects from exposure to plant toxins are: reduced growth, reduced production of eggs (Devanaboyina *et al.*, 2007) and milk, reproductive effects (Gatta *et al.*, 2013; Wocławek-Potocka *et al.*, 2013; Mustonen *et al.*, 2014) and immunomodulation causing increased vulnerability to contagious diseases (Fink-Gremmels, 2012; Chenchen *et al.*, 2014). For the European situation, EFSA has carried out several risk assessments on toxic plants and plant toxins in food and feed as presented in Table 6.

Essential oils have many pharmacological activities and overdosing may lead to adverse effects (e.g. Busquet *et al.*, 2006; Eisenhut, 2007). For example, the alkenylbenzene eugenol, a component of thyme and many other essential oils, is hepatotoxic when ingested in large quantities (James *et al.*, 2005). Veterinary offices also recognise the hazard of exposure to FCs of cattle, goat and sheep (Scott, 2007), horses (Higgins and Snyder, 2006), and birds (Ivie, 1978). Animals show comparable symptoms as found in humans, either after chronic ingestion of certain FCs or after skin contact: photosensitization or antiviral effects (Cain *et al.*, 2010). Some FCs, like bergamottin, are strong inhibitors of cytochrome P450 enzymes and may as such interfere with the metabolism of other compounds, including pharmaceutical substances.

Knowledge gaps

Climate changes: the types of plants and concentrations of plant toxins can change due to climate changes. Increased salinity of soils, due to prolonged periods of draught or expansion of cultivation land, can lead to accumulation of oxalate and coumarin in plants (Masters *et al.*, 2007). Furthermore, yearly variations in climate will influence the abundance of certain plants in a region and thus increase risks in certain periods (Cook *et al.*, 2015a). Worldwide, an increased occurrence of several weeds such as locoweed (Chenchen *et al.*, 2014) and Parthenium hysterophorus (Macconnachie *et al.*, 2010) is observed which results in a spread of the accompanying risks.

Table 7: Examples of several acute intoxications of farm animals[1] due to plant toxins

Animal	Symptoms	Plant related	Toxin related	Country	Reference
Farm animals	Acute renal failure, gastrointestinal signs and cardiac dysrhythmias	Oleander (*Nerium oleander*)	Oleandrin and oleandrigenin	USA	Kozikowski *et al.*, 2009; Forero *et al.* 2011
Cattle sheep horses	Feed intake reductions, loss of weight and fertility	Locoweed (*Astragalus* spp. and *Oxytropis* spp.)	Swainsonine, (trihydroxy indolizidine alkaloid)	China	Chenchen *et al.*, 2014
Farm animals	Neurological disease	*Ipomoea carnea* (fungal endophyte)	Swainsonine and calystegines	South America	Cook *et al.*, 2015a; Lu *et al.* 2015
Sheep	Neurologic disease	*Ipomoea asarifolia*	Swainsonine	World	Kleber *et al.* ,2014
Cattle	Cattle collapse, can be fatal	Anderson Larkspur -*Delphinium andersonii*	Diterpenoid alkaloids	USA	Pfister *et al.*, 2011
Cattle	Crooked calf syndrome; Fetal movement stops, causing the fetus to grow in contorted positions	Velvet Lupin – *Lupinus leucophyllus* Douglas ex Lindl.	Anagyrine alkaloid (teratogenic)	USA	Ralphs *et al.*, 2011
Sheep	Acute respiratory distress	*Galega officinalis*	Unknown	Belgium	Dierengezondheidszorg_ Vlaanderen, 2013
Camel, Goat, Sheep, Cattle	Bloating, diarrhea, stunted growth	*Pavetta gardeniifolia*	Not described	Africa	Abebe *et al.*, 2012
Farm animals	Blindness, convulsions, death	*Solanum* spp.	Solanine	World	Abebe *et al.*, 2012
Goat, Sheep, Cattle	Death	*Acokanthera schimperi*	Cardiotoxic glycoside ouabain.	Africa	Schmelzer and Gurib Fakim, 2008
Farm animals	Death	*Amsinckia* spp., *Senecio japobaea*, *Senecio* spp.	Pyrrolizidine alkaloids	North America	Forero *et al.*, 2011
Farm animals	Nervousness, muscle weakness, paralysis, death	*Delphinium* spp.	Diterpenoid alkaloids	North America	USDA, 2015 Cook *et al.*, 2015b
Farm animals	Hypocalcaemia, nephrotoxic, death	Sarcobatus vermiculatus, Oxalis spp., *Rumex* spp., *Amaranthus* spp., *Rheum* spp.	Oxalate	North America South America	Forero *et al.*, 2011

Animal	Symptoms	Plant related	Toxin related	Country	Reference
Goat, Sheep, Cattle	Death	*Triglochin* spp.	Unknown	North America	Forero *et al.*, 2011
Farm animals	Coordination disorders, death	*Prunus virginiana, Prunus serotina, Acroptilon repens, Centaurea solstitalis, Triglochin* spp.	Cyanogenic glycosides	North America	Forero *et al.*, 2011
Goat, Sheep, Cattle	Death	*Lolium* spp.	Alkaloids	World	Forero *et al.*, 2011
Sheep	Death, azotemia, hypocalcaemia	*Cicuta douglassi*	Cicutoxin (a neurotoxin)	North America	Reza Aslani *et al.*, 2011
Cattle	Teratogenic effects, reproductive effects	*Lupine* spp.	Piperidine alkaloid, Quinolizidine	North America	Lee *et al.*, 2006
Farm animals	Vertigo, vomiting and collapse	Cassava	Linamarin (cyanogenic glycosides)	World	FAO, 1990
Farm animals	Death	*Colchicum autumnale*	Alkaloids	Europe	Winter *et al.*, 2011
Farm animals	Anorexia, ruminal indigestion, edema in lips, tongue and face	*Centhatherum brachylepis*	Unknown	South America	Medeiros *et al.*, 2009
Horses	Anorexia, sleepiness, ataxia, weakness, stumbling	*Indigofera lepesdezoides*	Indospicine	North America, South America	Lima *et al.*, 2012
Goat, Sheep, Cattle, Pigs	Nervous signs, hypersensitivity, restlessness, stumbling gait, tremors, recumbence, tetanic and clonic convulsions, opisthotonos, teeth grinding, dyspnea, salivation, vomiting, death	*Marsdenia hilariana, M. megalantha*	Glycosides and Alkaloids	South America	Pessoa *et al.*, 2011

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[1] Farm animals include cattle, horse, sheep, goat and camel

Conservation techniques must be further developed to enhance nutritional quality by decreasing toxicity and anti-nutritional factors in the existing and newly available feeds, e.g. in cottonseed (Zhang *et al.*, 2007) and canola meal (Plaipetch and Yakupitiyage, 2013).

Risk assessments are required to accurately characterize the types of hazards and the dose–effect relationships. Moreover, adverse effects observed after consumption of feed materials by the animal could be associated with one or more plant toxins but can also be caused or enhanced by the accompanying mix of other undesirable substances (Fink-Gremmels, 2012). Therefore, the conclusion that was drawn in 2008 still stands (FAO and WHO, 2008).

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Veterinary drugs

Description of the hazard

Veterinary drug residues in animal feed are an issue for public, animal and environmental health for several reasons such as their toxicity and other impacts including the induction of resistance against veterinary antibiotics in target organisms (the latter discussed in section 3.2.5). The following topics emerged from the literature search and expert feedback as being of interest:

- Residues of antibiotics (i.e. agents that kill or inhibit the growth of micro-organisms, in this particular case chemical agents) in feed;
- Alternative practices to the use of antibiotics, including the use of prebiotics, probiotics, herbs, organic acids, livestock management practices and feed processing practices;
- Other veterinary drugs than antibiotics, such as anthelmintics with approved prophylactic uses against parasitic worms in livestock and non-antibiotic coccidiostats against cocccidiosis (Plasmodium infection) in poultry. While these groups are relevant and the issues surrounding their presence in feed resemble those with antibiotics, the emphasis in recent literature appears to rest with antibiotics;
- Illegal use of veterinary drugs, as this is a fraud-related issue, hence not within the remit of this report;
- Different regulatory approaches towards certain substances such as hormones and also differences in thresholds and/or substance classifications, which are, however, not within the scope of this paper given that they fall within the risk management and policy areas.

Veterinary drugs

Hazard	Veterinary drugs	
Source(s)	Anthropogenic (therapeutics) and natural (produced by micro-organisms) Cross-contamination from medicated feeds (e.g. accidental, in production plant) Feed produced from animals or plants previously exposed to antibiotics Wastewater and excreta contaminated with residues, exposure of cultured fish Antibiotics used in processes of which products can be used as animal feed (e.g. industrial fermentation processes such as for biofuel production from agricultural commodities)	
Transfer to food	Transfer: Usually high for kidney and liver of terrestrial animals (withdrawal times not respected in case of accidental feed contamination); Variable (low-high) for other edible tissues and food products (e.g. milk, eggs).	Strength of evidence: Strong
Potential impact on human health	Potential impact: Low for most antibiotics, low for coccidiostats	Strength of evidence: Strong
Potential impact on animal health	Potential impact: Low for antibiotics and Low to Medium for coccidiostats (e.g., toxic to horses)	Strength of evidence: Strong
Knowledge gaps	Uptake of environmental residues in e.g. crop plants. Impact on livestock gastro-intestinal microflora composition and traits (e.g., pathogen virulence). Risk of allergic/hypersensitive reactions (sensitization and elicitation) towards residues of antibiotics in animal products from non-target animals fed adventitiously contaminated feed (e.g., levels in such products as compared to elicitation thresholds).	

Sources

While medicated feeds and deliberate addition of antibiotics for growth-promoting effects extend beyond the scope of this report, other causes may motivate the presence of antibiotic residues in feed. These residues may be caused accidentally, such as through cross-contamination of non-medicated feed in facilities producing medicated feeds [e.g. (Stolker, Manti, Zuidema *et al.*, 2013)], or through the inclusion of contaminated feed ingredients, for example from animals exposed to antibiotics. In these cases, the drug residues are to be considered a class of contaminants rather than substances used for medicinal purposes. Other examples include the use of waste milk (from cows previously treated with antibiotics) as calf feed (Aust, Knappstein, Kunz *et al.*, 2013), or offal from antibiotic-treated cultured fish being fed to other aquaculture fish (Gill, 2000). Another important, emerging source of antibiotic residues in feed materials could be the use of antibiotics in fermentation processes for biofuel production (covered in section 4.3). These processes may give rise to residue in the derived by-products used for feed. Another practical example that is relevant to this topic is the use of wastewater and excreta, which potentially contain antibiotics or antibiotic-resistant microbes, as feed for aquaculture fish (Pruden, Larsson, Amezquita *et al.* 2013; Sapkota, Sapkota, Kucharski *et al.*, 2008), or as fertilizer for plankton or plants consumed by animals, such as in integrated pig-fish aquaculture systems (Rahman, Huys, Kuhn *et al.*, 2009; Son, Petersen, Truong *et al.*, 2011). This could in theory also be extrapolated to, for example, other systems where nutrients are recycled in a similar way between different livestock species, culture systems or trophic levels.

Awareness is also increasing about the natural occurrence of some antibiotics [e.g. chloramphenicol (Berendsen, Pikkemaat, Römkens *et al.*, 2013)] giving rise to background levels in feed, and hence also potential residues in food of animal origin. In more detail, these authors observed that when Streptomyces venezuelae was added to experimental lots of soil, it was capable of producing substantial amounts (>100 µg/kg) of chloramphenicol. In greenhouse-grown maize and wheat plants growing in pots with chloramphenicol-enriched soil, uptake of this compound was observed with residues particularly occurring in plant stems, indicating that straw and other crop products used as bedding or feed may serve as natural sources (Berendsen *et al.*, 2013). This has been further confirmed by Nordkvist *et al.* (Nordkvist, Zuidema, Herbes *et al.*, 2016) who observed substantial levels of chloramphenicol in real-life samples of straw obtained from various European countries and different cereal species. Chloramphenicol may not be an exception, though, given that the majority of antibiotics developed for human and veterinary purposes are based on naturally occurring compounds [e.g. reviewed by Nicolaou *et al.* (Nicolaou, Chen, Edmonds *et al.*, 2009)]. Any new or emerging risks posed by an antibiotic used for veterinary purposes which is also formed in nature should therefore be offset against the risk linked to the background exposure caused by its natural occurrence.

Transfer from feed to food of animal origin

During the pre-market risk assessment of antibiotics and veterinary drugs, the potential transfer of these drugs to edible animal tissues is commonly considered. Based on the observed transfer, metabolism and toxicity of these residues under conditions of their prospective usage, safe and technically feasible maximum residue limits (MRLs) for such residues will be established according to internationally harmonized principles as employed, for example, by the FAO/WHO Joint Expert Committee on Food Additives [JECFA; e.g. (IPCS, 2009)]. Besides regulatory studies, also various scientific studies published in literature have observed residues resulting from transfer of antibiotics from medicated feed, for example, to muscle tissue in aquacultured carp [oxytetracycline (Elia, Ciccotelli, Pacini *et al.*, 2014)]. Similar to therapeutically used antibiotics, it can be envisaged that residues of the same antibiotics adventitiously present in feed could be transferred to edible tissues as well. As a model for cross-contamination with medicated feed, Vandenberge *et al.* (Vandenberge, Delezie, Delahaut *et al.*, 2012a, Vandenberge, Delezie, Delahaut *et al.*, 2012b) observed, for example, transfer of flubendazole to breast and thigh of broiler chicken and to eggs from laying hens both fed diets containing flubendazole at levels up to 10% of therapeutic levels, while no such transfer was observed for tylosin. In several other experiments with various antibiotics and coccidiostats, such transfers caused the transient build-up of substantial residue levels for some but not all of the compounds tested, such as for sulfadiazine, doxycycline, and lasalocid in poultry (Vandenberge, Delezie, Huyghebaert *et al.*, 2012a; Vandenberge, Delezie, Huyghebaert *et al.*, 2012b).

Potential impact on human health

As stated in the previous paragraph, potential health implications of veterinary drug residues in animal-derived food products after transfer from feed are commonly considered during the regulatory pre-market assessment of these drugs. As a result, dosage regimes, withdrawal times, and maximum residue levels are usually established accordingly with the aim of protecting consumer health. In case of adventitiously cross-contaminated feed, these provisions may not be abided by and the feed may also be consumed by non-target species. The residues are likely to be relatively low, for which reason allergic and other hypersensitivity reactions may be the primary concern, particularly in individuals that have already become sensitized. Especially antibiotics such as penicillin and other beta-lactams, are known to cause such reactions in patients, for example through consumption of milk containing antibiotic residues (Katz and Brady, 2000). This risk of allergic reactions is considered by medicinal registration agencies and JECFA when setting threshold levels for residues of penicillin and other antibiotics in animal-derived food products during registration of these antibiotics as veterinary drugs (EMA, 2009; Woodward, 2004). Obviously, the occurrence of antibiotics in edible products from non-target animals fed adventitiously contaminated feeds would not fall within the remit of such regulatory assessments, and therefore it would be useful to be able to assess the likelihood of allergic reactions in these cases as well.

For the potential presence of coccidiostat residues in feed for non-target animals through potential cross-contamination from medicated feed production, the potential health implications for human consumers were reviewed by Dorne *et al.* (Dorne, Fernandez-Cruz, Bertelsen *et al.*, 2013). Consumer exposure estimates were below the acceptable daily intake (ADI) for the wide range of coccidiostats considered, with the highest proportion (97%) of the ADI reached by exposure to halofuginone in consumed liver, kidney, muscle, skin and fat of non-target animals. These authors concluded that the coccidiostat residues would not be expected to cause health risks (Dorne *et al.*, 2013).

Potential impact on animal health

While the potential toxicity of therapeutic doses of antibiotics administered during a limited period is commonly addressed as part of their pre-market risk assessment, this scenario may be different from that of lower-level adventitious presence of antibiotic residues, particularly if the latter occurs over a prolonged period similar to the addition of antibiotics to feed for growth promotion. The growth-promoting effects of such residues at sub-therapeutic levels are considered to be linked to impacts on the intestinal microflora, more particularly by reduction of the formation of growth-suppressing metabolites and decreased numbers of bacteria competing for nutrients, among other things (Allen and Stanton, 2014). Conversely, intestinal bacteria that are antagonistic to intestinal pathogens may in some cases also be suppressed (Allen and Stanton, 2014), while virulence genes other than antibiotic resistance genes in pathogens may be up- or down-regulated by the low-level presence of antibiotics depending on the specific antibiotic and pathogen involved (Andersson and Hughes, 2014).

As regards coccidiostats, the narrow margin between therapeutic and toxic doses of these compounds in some animal species, particularly poultry (Dorne *et al.*, 2013), may be of concern. Moreover, differences among livestock animal species towards the toxicity of these compounds will be of concern if non-target animals are exposed to coccidiostats, particularly a sensitive species like horses.

Final remarks

While the focus of many publications in this field is on antibiotics, various topics have also been identified in recent literature which can also be briefly mentioned here. For example, a topic of recent interest includes residues of inorganic therapeutics [e.g. arsenic-based drugs (FDA, 2015)]. Another recent trend is the search for appropriate alternatives to veterinary antibiotic usage (Huyghebaert, Ducatelle and Immerseel, 2011). This follows the reduced use or prohibition of such antibiotics used as of growth-promotors, or to treat infectious bacterial diseases (Pruden *et al.*, 2013). Examples of such products added to feed include essential oils, peptides, organic acids, enzymes, vaccines, pre- and probiotics (Huyghebaert *et al.*, 2011). For some of these alternatives, scrutiny for potential transfer of chemical residues to animal-derived food products is warranted.

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BIOLOGICAL HAZARDS

Bacterial agents

There is a continuous risk for (micro-) biological contamination of animal feed throughout the production chain, from the growing period in the field up to the moment when fed to the animals. Such diverse risks for contamination make it difficult to control or fully eliminate specific pathogens. The ultimate objective is, therefore, to produce feed where the microbial contamination is generally kept at such a level and, in particular so for specific pathogens, to ensure that animals fed do not become infected and colonized with the risk for subsequent spread, e.g. within a herd, in the environment or ultimately to consumers.

Hazard Analysis Critical Control Point (HACCP) programs and Good Agricultural Practices (GAP) are utilized to control pathogenic agents in animal feed. In order to identify and characterize pathogens, understanding the factors that affect the sources and routes of contamination alongside the occurrence of contaminated feed and feed ingredients remains crucial in order to prevent further contamination. Depending on the feed manufacturing process, opportunities for reduction of bacterial hazards may be limited e.g. if there is no heat-processing step. Nevertheless, for industrialized feed production, which produces large volumes of feed that are often distributed widely, particular attention is needed to minimize the potential to introduce new hazards into a food production system via such feed.

Since the FAO and WHO (2008) report, a European Food Safety Authority (EFSA) opinion on the microbiological risk assessment in feedingstuffs for food-producing animals was published. In this report, experts identified *Salmonella* spp. as the main hazard for microbial contamination in animal feed (EFSA, 2008). Subsequently, a 2012 comprehensive overview on animal feed contaminations has also highlighted *Salmonella* spp. as the major microbiological hazard in animal feeds (Liebana,Hugas and Fink-Gremmels, 2012). In accordance with the EFSA (2008) opinion and the assessment of microbiological risks from Liebana *et al.* (2012), biological hazards of interest in this section include *Salmonella* spp. and to a lesser extent other pathogenic bacteria.

Potential sources of pathogens in animal feed can occur throughout the production chain; these sources are briefly outlined. For example, feed contamination, being either direct or indirect, may result from raw ingredients, soil, irrigation water, or manure. The manufacturing and processing of feed ingredients and feed, e.g. in crushing plants and feed mills has been reported as a source of contamination and cross-contamination from the facility and between different feed sources/ingredients may occur (Alali,Ricke and Fink-Gremmels, 2012, Jones, 2011). Processing of feed materials, whether they are of plant, fish or animal origin, typically involves processing steps like heat and/or chemicals followed by cooling. In the case of insufficient hygiene measures or controls and with inefficient cooling conditions, the growth of certain pathogenic microorganisms, like *Salmonella*, may occur (Binter,Straver,Haggblom *et al.*, 2011). Furthermore, transports between these locations can affect the survival and growth of pathogens; for example, due to poorly cleaned, or unclean trucks that may have previously held contaminated feed, or because of uncontrolled temperature or humidity settings during feed transport (Alali *et al.*, 2012). On this point, environmental sources of pathogenic feed contaminations such as the poultry houses and poultry litter (e.g. with *Salmonella*) as well as the possibility of faecal shedding during cattle entrance to the feedlots and manure handling practices have been noted (e.g. for *E. coli* O157:H7) (Alali *et al.*, 2012, Frederick and Huda, 2011).

In general, there is limited evidence to support the notion that spread of bacterial infections through animal feed is considerable; notable exceptions include *Salmonella* spp. (Crawshaw and Fink-Gremmels, 2012, EFSA, 2008, Jones, 2011, Liebana *et al.*, 2012). Hence, *Salmonella* spp. are emphasized, while other relevant feed-related pathogenic microorganisms like *Listeria* spp., *Escherichia coli* (STEC), *Clostridium* spp., and *Mycobacterium* spp. are briefly highlighted. To correlate with the FAO and WHO (2008), *Brucella* spp. are also covered, although new information is limited. Finally, the notion of antibiotic resistance is addressed as the possible role that antibiotics play in animal feed to the resistance of intestinal bacteria deserves further attention.

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Salmonella spp.

Description of hazard

Salmonellosis is the infection caused by pathogens of the genus *Salmonella* and is recognized as one of the most important causes of foodborne illnesses (Adams and Moss, 2008, Scallan, Hoekstra, Angulo *et al.*, 2011, World Health Organization (WHO), 2016). More than 2,500 *Salmonella* serotypes, or serovars, have been reported, yet many of them rarely cause foodborne illness (Grimont and Weill, 2007). This may be due to the variation of microbial pathogenic determinants at species and strain level, which may affect the severity of illness.

The issue of strain variation and their impact on *Salmonella* survival in feed, as well as in low moisture foods, has highlighted the dominance of some strains in dry environments (Burns, Duffy, Walsh *et al.*, 2016). Andino, Pendleton, Zhang *et al.* (2014) highlighted that exposure to stress in dry (poultry) feed could increase the persistence of S. enterica and enable survival for long periods.

Sources

The prevention of pathogens is critical throughout the food chain and control measures can help to minimize transmission routes. Adams and Moss (2008) indicate that food animals may acquire *Salmonella* infections on the farm from wild birds and rodents;

Salmonella spp.

Hazard	***Salmonella* spp.**	
Source(s)	Contaminated feed material of oil seed or fruit origins, of marine origin (fish meal), or land animal origin (meat (and bone) meal); Environmental sources such as poultry houses and poultry litter; Breeder and slaughter animals including asymptomatic carriers (swine, poultry, and cattle); wild birds and rodents; crushing and feed producing plants especially when hygiene controls are insufficient since this may allow pathogenic growth.	
Transfer to food of animal origin	**Potential transfer:** high	**Strength of evidence:** Strong
Potential impact on human health	**Potential impact:** high	**Strength of evidence:** Strong
Potential impact on animal health	**Potential impact:** high	**Strength of evidence:** Strong
Knowledge gaps	**Data:** The lack of targeted studies to better estimate "feed as a source of introduction of new serovars of *Salmonella* into animal herds" (Wierup, 2013). The source of why and where *Salmonella* contamination occurs in soybeans and how to prevent them is limited; a preventative approach to minimize the spread in feed and food chains is required, and authors point to eliminating contamination at the crushing plant as a step (Wierup and Kristoffersen, 2014). The lack of harmonized sampling data between different countries makes it difficult to quantify the risk. **Methodologies:** A need for sampling methods for the design of a robust sampling protocol for substantiating *Salmonella*-free in feed or feed ingredients (Wierup, 2013). A need for an adjustment in methodologies to enable quantification and/or detection of *Salmonella* in feed given current situations (uneven distribution of cells, harsh environments, etc.) (Schelin, Andersson, Vigre *et al.*, 2014).	

however, principal sources are other animals, including asymptomatic carriers and contaminated feedingstuffs. Therefore, *Salmonella*-safe feed is necessary to prevent further contamination in breeding animals (Fedorka-Cray, Kelley, Stabel *et al.*, 1995).

According to a quantitative risk assessment, the two principal sources of *Salmonella* infection in breeder and slaughter pigs were infected incoming pigs and contaminated feed (European Food Safety Authority (EFSA), 2010). Moreover, Lewerin, Boquist, Engström *et al.* (2005) reported a similar situation for poultry. Further, Amagliani, Brandi and Schiavano (2012) reviewed the influence of *Salmonella* in seafood safety and indicated that the presence of *Salmonella* in seafood might result from contaminations in the natural aquatic environment, presumably through faecal contamination, from aquaculture, or during processing.

Moreover, the processing of feed materials, whether they are of plant, fish or animal origin, typically involves processing steps like heat and/or chemicals followed by cooling. In the case of insufficient hygiene measures or controls and with inefficient cooling conditions, the growth of certain pathogenic microorganisms, like *Salmonella*, may occur (Binter, Straver, Haggblom *et al.*, 2011). Recently, Gong and Jiang (2017) investigated the potential for cross-contamination of *Salmonella* in rendering processing environments, observing that the receiving area, surfaces surrounding cracks grinding, and finished meal loading-out areas were areas of concern; notably, the same *Salmonella* serotypes were found in the raw materials receiving area and the finished meal loading-out areas suggesting possible cross-contamination. Due to limitations in the cleaning with water in this dry environment, measures that support a good hygienic design, which can, e.g. prevent harbourage of pathogens or biofilm formation, are needed. The survival of particular clonal groups of *Salmonella* at feed mills and feed factory environments has been reported (Pellegrini, Paim, de Lima *et al.*, 2015; Prunić, Milanov, Velhner *et al.*, 2016).

Besides this, an overview from Davies and Wales (2013) highlights the issue of storage conditions, of cereal ingredients for animal feeds, as a source of contamination with *Salmonella*. Particularly, the use of areas where livestock had been kept as temporary storage locations for grain instead of, e.g. storing grain directly in main storage bins or at least 1 km from the livestock accommodations is noted. Also, the effect of wildlife and domestic animal access to grain were described. Consequently, the effect that the shared use of inadequately cleaned equipment and the opportunities for farm access that wildlife and animals had, played a role in the environmental contamination reported.

The Centers for Disease Control and Prevention (CDC) has made available an Atlas of *Salmonella* surveillance data from the United States (1968-2011), which includes data on isolates from animals and related sources such as the environment and feed. Within this atlas, the percentage of non-human isolates by type (clinical or non-clinical) and source (including feed/feed supplements), as reported by the National Veterinary Service Laboratory (NVSL) from 1968-2011, were evaluated for the top 30 serotypes determined during this period. In brief, the top three *Salmonella* serotypes coming from feed/feed supplements for clinical cases was that of Oranienburg (19.69%), Senftenberg (10.33) and Schwarzengrund (10.03%), while for non-clinical cases this was Berta (1.20%), Muenchen (0.79%), and Oranienburg (0.66%) (Centers for Disease Control Prevention (CDC), 2013). However, the CDC states that these data warrant caution as sampling was neither complete nor representative (Centers for Disease Control Prevention (CDC), 2013). Regarding organic feed, the presence of *Salmonella* in certified organic feed was found to be significantly lower than in conventional broiler feed (Alali, Thakur, Berghaus *et al.*, 2010). Further, the survival of *Salmonella* in certified organic and conventional feed did not significantly differ (Petkar, Alali, Harrison *et al.*, 2011).

In a joint EFSA and European Centre for Disease Prevention and Control (ECDC) scientific report summarizing European Union (EU) trends of zoonoses, zoonotic agents, and foodborne outbreaks in 2013, feedingstuffs, in the context of *Salmonella* contaminations, were noted for animal- and vegetable-derived feed material and compound feedstuffs. For vegetable-derived feed material, the highest proportion of positive samples was reported for feed materials of oil seed or fruit origin including rape seed-derived, soy(bean)-derived, sunflower seed-derived, and cotton seed-derived feed (European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC), 2015). Also, a moderate to high contamination was reported in feed material of marine origin, i.e. fish meal (Crump, Griffin and Angulo, 2002, European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC), 2015), and food of land animal origin (i.e. meat meal) (European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC), 2015). Collected Member State (MS) data from targeted surveillance programs noted that *Salmonella* contaminations are low with 1.4% positive samples from 15,315 tested for animal- and vegetable-derived feed material (European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC), 2015). Similarly, *Salmonella*-positive samples in compound feed were also low for cattle 1.8% of 1,091 tested samples, pigs 1.6% of 1,590 tested samples, and poultry 1.9% of 2,551 tested samples reported (European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC), 2015). The ten most commonly reported *Salmonella* serovars for feed from cattle, pig, and poultry (including breeding flocks, broilers and laying hens) in the EU had been reported. Feed for cattle ranked Infantis (54.6%) as the most commonly reported, while Senftenberg and Typhimurium ranked first and second for pig feed, at 22.2% and 16.7%, respectively, as well as for poultry feed at 19.5% and 17.1%, respectively (European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC), 2015). One point for attention is that these newer data from the EU/EFSA/ECDC on the successful prevention and control lack a harmonized sampling, meaning that the data should be treated with caution.

The reasons for the reoccurring presence and isolation of specific serovars has been thought to be the result of continuous contaminations at crushing and feed producing plants (Liebana, Hugas and Fink-Gremmels, 2012). For the fish meal, contamination may be related to rodents or birds (Adams and Moss, 2008).

Transfer to food of animal origin

Transmission of *Salmonella* in the animal feed to animals that consume the feed and also to food of animal origin has been documented (European Food Safety Authority (EFSA), 2008). In a review concerning *Salmonella* control measures in animal feed, Jones (2011) states that the link between animal feed and both human and animal salmonellosis has been long established despite researchers' questions about the relationship between *Salmonella* serotypes found in feed and those that commonly cause the disease.

Potential impact on human health

Salmonellosis occurs by ingesting contaminated animal food products (such as eggs and (poultry) meat), although green vegetables treated with contaminated manure may also cause transmission (World Health Organization (WHO), 2016). When ingested, non-typhoidal *Salmonella* spp. usually causes a mild disease, which may start 6 to 72 hours after infection. Typical symptoms are fever, diarrhoea, abdominal cramps, nausea and sometimes vomiting. Most people recover within 2 to 7 days without treatment. Nevertheless, 33 million healthy life years are lost as a result of salmonellosis. Moreover, in susceptible hosts, such as children and the elderly, salmonellosis may cause dehydration resulting in hospitalization or even death (World Health Organization (WHO), 2016). In the USA, *Salmonella* spp. is the most relevant pathogen with an estimated one million foodborne illnesses per year, 19,000 hospitalizations, and 380 deaths (Centers for Disease Control Prevention (CDC), 2016).

Globally, 1 in 10 people get salmonellosis each year: in total 550 million people, almost half of which are children. As a result, it is one of the four key global causes of diarrhoea. The seriousness of the disease depends on the susceptibility of the patient, but also on the serovar. Host-specific serovars, such as S. Dublin in cows and S. Choleraesuis in pigs, cause invasive disease in humans and can be life-threatening (World Health Organization (WHO), 2016). An increasing concern is the development of antimicrobial resistance of *Salmonella* spp. European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC) (2016) reported that high proportions of human *Salmonella* isolates were resistant to tetracyclines (30%), sulfonamides (29%), and ampicillin (28%). Some serovars had a very high multi-drug resistance, such as S. Kentucky (75%), monophasic S. Typhimurium (70%), and S. Infantis (62%) (European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC), 2016). In a comprehensive Irish feed mill study by Burns,Lawlor,Gardiner *et al.* (2015), *Salmonella* contamination, with monophasic variants of S. Typhimurium, in feed ingredients and compound feeds for pigs was observed at a low prevalence; nonetheless, even low levels can be of a concern for animal health and subsequent human infection with consumed contaminated pork. Moreover, all the strains recovered exhibited antibiotic resistance with some multi-resistant isolated found, thus indicating a further concern (Burns *et al.*, 2015).

Salmonella-contaminated animal feed has been reported to contribute to human infection as a result of the use of contaminated fish meal imported as feed material. Between 1968-1972, S. Agona occurred in the US and Europe, and since then it is a prevalent serotype in humans causing an estimated 1 million human illnesses, as of 2001, in the US alone (Crump *et al.*, 2002).

Strategies to prevent and to control *Salmonella* contamination are crucial. For example, Wierup and Kristoffersen (2014) reported that nine of the top ten EU serovars that were isolated from clinical cases of salmonellosis in humans were isolated from soybeans intended as feed ingredients, including major pathogenic *Salmonella* serovars like Typhimurium and Enteritidis.

Potential impact on animal health

Various *Salmonella* serotypes have been found in animal feed and were linked to salmonellosis in humans and animals (Jones, 2011). The relative contribution of feed to animal infection is region dependent meaning that there may be differences in the prevalence of serotypes, and therefore, the potential serotypes of concern.

Feed is the primary source of infection in regions with a low *Salmonella* prevalence status, whereas feed contributes less to the overall infection rate in regions with a high prevalence status (European Food Safety Authority (EFSA), 2008). For example in the EU, when monitoring *Salmonella* in the animal feed sector, five serovars have been reported as critical: Enteritidis, Typhimurium, Infantis, Virchow and Hadar (Liebana *et al.*, 2012) as these are mentioned in Regulation (EC) 2160/2003. These serotypes are particularly important for human health. Further in the USA, the FDA has provided examples of animal feeds and pathogenic *Salmonella* serotypes associated with salmonellosis (eight serotypes in five classes of food animals): "Poultry feed with S. Pullorum, S. Gallinarum, or S. Enteritidis; Swine feed with S. Choleraesuis; Sheep feed with S. Abortusovis; Horse feed with S. Abortusequi; and Dairy and beef feed(s) with S. Newport or S. Dublin" (Food Drug Administration (FDA), 2013). If such cases occur, this warrants FDA regulatory action.

When feed is contaminated with pathogenic serotypes, this usually causes colonization of the intestines and long-term shedding. Pigs and poultry are frequently infected, but infection is usually asymptomatic. *Salmonella* infection hardly causes problems in chicken, but turkey and pigs may be diseased. On the other hand, ruminants are less frequently infected, but when they are infected, the likelihood of disease is higher. Certain *Salmonella* serotypes are host-specific and cause predictable clinical signs. For example, S. Gallinarum causes fowl typhoid, and S. Pullorum causes pullorum disease in poultry, whereas S. Choleraesius causes enteritis and septicemia in pigs, S. Abortusovis causes abortion in sheep, and S. Dublin is associated with abortion, enteritis, and septicemia in cattle. The latter species is widespread in European livestock farming (Liebana *et al.*, 2012).

In conclusion, animals are frequently infected with *Salmonella*, but this rarely causes disease. The main problem is the asymptomatic carriage, which may cause the spread of infection to other animals, the environment, and humans (European Food Safety Authority (EFSA), 2008; Liebana *et al.*, 2012).

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Listeria monocytogenes

Listeriosis is a serious infection caused by eating food contaminated with *Listeria monocytogenes.*

L. monocytogenes is ubiquitous and human exposure is thought to be frequent, but the incidence of infection is low and dependent on individual susceptibility; the young, old, pregnant, and immunocompromised persons (YOPIs) are found to be more susceptible (Adams and Moss, 2008; CDC, 2015). In addition to the wide-distribution of *L. monocytogenes* in the environment, it is markedly known for its potential growth in non-acidic foods, which provides several opportunities for entrance and subsequent multiplication in the food chain (Adams and Moss, 2008). Some sources of *Listeria monocytogenes* include soil, sewage, forage, and water (Adams and Moss, 2008; EFSA, 2008). Concerning the presence of *L. monocytogenes* in food of animal origin, meats and meat products including cured meats, dairy products like unpasteurized, raw milk and soft cheeses, as well as prepared seafood like smoked fish are often associated with major outbreaks of listeriosis (Sikorski and Kalodziejska, 2002; Adams and Moss, 2008; CDC, 2015). Concerning unprocessed feeds, such as plant materials that either is not or is minimally processed, such as silage, *L. monocytogenes* can have the opportunity to grow out if production is not properly controlled. As with *Salmonella* spp., evidence of pathogen transmission in the feed is also reported for *L. monocytogenes* (EFSA, 2008). For example, Liebana *et al.* (2012) have noted the risk of *L. monocytogenes* in silage as related to animal listeriosis and asymptomatic carriage in dairy cattle, sheep, and goats. During a case-control study, Nightingale *et al.* (2004) observed that the epidemiology and transmission of *L. monocytogenes* differ between small-ruminant and cattle farms. Also, authors observed the prevalence of *L. monocytogenes* in clinical cases and fecal samples as being more frequent in environmental than feed samples; thus, indicating that infected animals may contribute to dispersal in the farm environment (Nightingale *et al.*, 2004). The International Commission on Microbiological Specifications for Foods (ICMSF) has recommended appropriate fermentation conditions for roughage use for silage to control *L. monocytogenes* (Swanson, 2011). *L. monocytogenes* has been detected in poultry feed both before

Listeria monocytogenes

Hazard	*Listeria monocytogenes*	
Source(s)	Ubiquitous: soil, sewage, water, feed (unprocessed feed, forage, silage), feed mill, contaminated foodstuffs (meats and meat products including cured meats, dairy products like unpasteurized raw milk and soft cheeses, and prepared seafood like smoked fish).	
Transfer to food of animal origin	**Transfer:** medium	**Strength of evidence:** moderate-weak
Potential impact on human health	**Potential impact:** high especially for YOPIs*	**Strength of evidence:** strong
Potential impact on animal health	**Potential impact:** high	**Strength of evidence:** strong
Knowledge gaps	The relationship between feeding poor quality silage and eventual listeriosis in animals warrants further research. In addition, further studies that investigate the mechanisms of climate stress and animal health in relation to listeriosis outbreaks may be required.	

* Young, old, pregnant and immunocompromised persons (YOPIs)

and after heat treatments. The feed mill has been suggested as a source of *L. monocytogenes* indicating potential re-contamination of pellet feed (Liebana *et al.*, 2012). However, the prevalence of *L. monocytogenes* in animal feed is believed to be low in ingredients with a low water activity such as hay and cereal grains. McLauchlin *et al.* (2014) noted a strong relationship between feeding poor-quality silage and listeriosis in sheep and cattle, yet cases also were observed in the absence of this feed type. The mechanisms by which silage-feeding leads to listeriosis is not clear. Furthermore, outbreaks have been associated with climate stress and observed in the spring when animals may be in a weak condition or exposed to poor-quality feed (McLauchlin *et al.*, 2014).

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Shiga Toxin Producing Escherichia coli (STEC)

Some *Escherichia coli* (*E. coli*) are pathogenic and cause human illnesses; among these *E. coli* O157:H7 is one of the most common enterohemorrhagic *E. coli* (EHEC) reported. EHEC is a strain of *E. coli* that produces a Shiga toxin. Shiga-toxin producing *E. coli* (STEC), also known as verotoxin-producing *E. coli* (VTEC), is a foodborne zoonotic agent of concern to public health. STEC can be transmitted via the faecal-oral route meaning cross contamination, e.g. via feed, water, food, or environmental sources, as well as contact with infected animals are relevant sources (Adams and Moss, 2008; WHO, 2011; CDC, 2014). STEC infections can affect people of all ages. However very young children and elderly persons are more susceptible to developing severe illnesses like haemolytic uremic syndrome (HUS) (WHO, 2011; CDC, 2014). Concerning the transfer of *E. coli* O157:H7 to food of animal origin, meat products (e.g. especially undercooked ground meat) and unpasteurized, raw milk or soft cheeses made from raw milk are recognized as foods that are considered to carry a high risk of infection (Adams and Moss, 2008; WHO, 2011; CDC, 2014). These food sources are concurrent with previous outbreaks of *E. coli* O157:H7 in the USA and the UK; such outbreaks were attributed to fundamental errors in food hygiene concerning heat processing and adequate cooking (Adams and Moss, 2008). Cattle have been reported as an important reservoir of O157:H7 infection (Adams and Moss, 2008). *E. coli* O157:H7 has rarely been detected in cattle feed; however, some literature suggests that feed may be a source of *E. coli* O157 in cattle (Alali *et al.*, 2012). Dodd *et al.* (2003) specifically note that further studies on the prevalence of *E. coli* O157 in cattle feed are warranted. Hutchison *et al.* (2007) had investigated time/temperature combinations that were applied in commercial pelleting processing and concluded that these would not effectively kill high *E. coli* O157 numbers in the feed (Hutchison *et al.*, 2007; EFSA, 2008; Liebana *et al.*, 2012). Furthermore, Callaway *et al.* (2009) had evaluated some studies on the

Escherichia coli

Hazard	*Escherichia coli* (Shiga-toxin producing *E. coli*, STEC)	
Source(s)	Cattle, pigs, birds, herbivores (e.g. as carriers), pasture grass, feed (silage), water, food (e.g. unpasteurized milk, undercooked ground beef, contaminated raw produce).	
Transfer to food of animal origin	**Transfer:** medium-low	**Strength of evidence:** moderate-weak
Potential impact on human health	**Potential impact:** high	**Strength of evidence:** strong
Potential impact on animal health	**Potential impact:** high for young animals; low for adult animals*	**Strength of evidence:** strong
Knowledge gaps	Scientific data on the potential transfer has indicated that limited transfer of the agent from feed to food may occur. The effect of (cattle) diet (feedstuffs) on the prevalence of STEC, appears to alter shedding levels, although further research to support these findings is warranted. The literature on experimental studies supporting the potential strength on the uptake/transfer of the hazard from consumed feed in livestock or other, relevant animal model species is limited, although some literature suggests there may be a potential link. The prevalence of *E. coli* O157, among other STEC, in cattle feed requires further research.	

*Food safety issues relate to the fact that adult animals do not suffer major impacts, yet act as carriers. In cattle, the prevalence of STEC was significantly higher in calves than in adult cattle (Jaros *et al.*, 2016).

effects of diet in cattle and *E. coli* O157:H7 populations concluding that feedstuff appears to alter shedding levels, but effects were inconsistent. In general, a combination of fasting and a poor quality forage diet had shown to increase shedding of *E. coli* O157:H7 in cattle with a noticeable reduction in generic *E. coli* and *E. coli* O157:H7 populations upon switching cattle from a grain ration to high quality hay based diet (Callaway *et al.*, 2009). Nonetheless, this change could also be attributed to reduced animal health. Similarly, Fenlon and Wilson (2000) and Duniere *et al.* (2011) both reference the importance of proper silage conditions to prevent survival of STEC, which can be found in pasture grass. More recently, Callaway *et al.* (2013) reviewed STEC ecology in cattle management including potential control and intervention methods, such as diet and water management alongside other pre-harvest processing strategies, in order to reduce STEC in faecal shedding. Authors acknowledged that pre-harvest controls could help to reduce, and thus manage STEC, however complete elimination of STEC based on such controls is unlikely, and accordingly, procedures during processing are still required. In short, a multi-hurdle, live-animal intervention approach that complements in-plant investigations should be maximized in order to achieve an acceptable reduction of the pathogen to the supply chain (Callaway *et al.*, 2013).

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Clostridium spp.

Clostridium spp. consist of gram positive, spore forming bacteria that have been identified as a concern in feedingstuffs for food-producing animals, yet to a lesser extent as compared to *Salmonella* spp. (EFSA, 2008; Liebana *et al.*, 2012). Nevertheless, it is also important to note that clostridal toxins and spores cannot be easily neutralized through heat and pressure treatments of feeds, meaning further contamination can result during the compounding process (EFSA, 2008).

Clostridium perfingens is ubiquitous, being found in soil, contaminated food (e.g. raw meat and poultry), decaying vegetation, marine sediment, the intestinal tracts of birds, poultry litter, as well as the intestines of human and animals (Adams and Moss, 2008; CDC, 2014; Udhayavel *et al.*, 2017). *C. perfringens* has been commonly reported in feces and soil; due to its possible presence in soil-contaminated feeds, it is reported as a component of feed and can be present as either vegetative cells or endospores (EFSA, 2008; Liebana *et al.*, 2012). *C. perfingens* type A has shown to cause necrotic enteritis in poultry (Udhayavel *et al.*, 2017). A recent study observed that from 298 samples analyzed for *C. perfingens* from poultry feed ingredients, 101 were positive (33.9%); the highest numbers of positives were detected in fish meal (55.3%), bone meal (44.8%), meat and bone meal (42.7%), and dry fish (38.5%) (Udhayavel *et al.*, 2017). Although *C. perfringens* is commonly isolated from the environment and the intestinal tracts of broilers, its significance in feed contamination has been questioned as *C. perfringens*-associated diseases need initiators in addition to microorganism presence (EFSA, 2008; Liebana *et al.*, 2012). Nevertheless, *C. perfingens* is considered one of the most common causes of foodborne illnesses (Adams and Moss, 2008; CDC, 2014). Food poisonings due to *C. perfingens* can arise in food of animal origin when meat or poultry is poorly prepared (e.g. undercooked) or when spores survive the cooking processes. Also, if cooked food is improperly stored (e.g. prolonged storage at room temperature), spores can germinate and rapidly multiply (Adams and Moss, 2008; CDC, 2014).

Clostridium spp.

Hazard	*Clostridium* spp.	
Source(s)	Ubiquitous: soil, decaying vegetation, marine sediment, poultry litter, feed (silage, haylage, grain), carcass contamination of feed, contaminated foodstuffs (raw meat, poultry, dairy), intestinal tract of human and animals.	
Transfer to food of animal origin	**Transfer:** medium-low	**Strength of evidence:** moderate-weak
Potential impact on human health	**Potential impact:** high	**Strength of evidence:** strong
Potential impact on animal health	**Potential impact:** high	**Strength of evidence:** strong
Knowledge gaps	Fecal and environmental contamination, e.g. at dairy farms, of *C. botulinum* may pose a risk later in the dairy production chain. For example, the prevalence of *Clostridium* spores (e.g. in raw milk) is unknown, yet the transmission thereof can be of concern to human health (Lindström *et al.*, 2010). The relationship between contaminated animal feed and C. perfringens-associated diseases should be further investigated. Reliable methods to detect *C. botulinum* toxin in animal feed are lacking.	

Seven types of *C. botulinum* exist (A-G) of which A, B, E, and F can cause disease in humans, with A, B, and E associated with foodborne illness. Types C and D are generally associated with causing disease in animals, while no disease is associated with type G (previously *C. argentinense*) (Public Health Agency of Canada, 2011). *C. botulinum* can be found in the soil and grows well at micro-aerobic conditions (Adams and Moss, 2008). *C. botulinum* intoxications have been reported in cattle and equine with common sources originating from the toxins produced by contaminating *C. botulinum* bacteria in silage, haylage, i.e. round bale silage, and grain of poorer quality. Lindström *et al.* (2010) reported that the use of non-acidified and plastic-wrapped silage led to an increase in botulism outbreaks, resulting from *Clostridium* botulinum, in dairy cattle. Bodies of small animals such as lizards, snakes, turtles, rodents, birds and cats that are inadvertently trapped in silage, hay or grain during harvest or storage are some of the common types of rotting organic matter that contaminate feed (Galey *et al.*, 2000; Myllykoski *et al.*, 2009; Lindström *et al.*, 2010). For example, Myllykoski *et al.* (2009) reported a botulism outbreak in Finland where nine out of 90 dairy cattle died after being fed with non-acidified silage contaminated with animal carcasses; the type *C. botulinum* neurotoxin genes and neurotoxin itself were identified. High moisture feeds such as silage or brewer's grains, when allowed to rot rather than ferment, can also provide an ideal anaerobic environment for botulism growth (Freeman and Bevan, 2007). Another source may be the use of contaminated poultry litter that has been spread onto cattle pastures (EFSA, 2008; Liebana *et al.*, 2012). Also, ensiling of forage harvested too short after application of manure could increase the potential for *C. botulinum* (Lindström *et al.*, 2010). Growth of these bacteria is possible within silage under unfavorable fermentation conditions and when pH is high, generally over 4.5 (Wong *et al.*, 1988). Overall, the direct source of *C. botulinum* can be misconceived; however, the cause of botulism in animals is connected to the presence and multiplication of the *C. botulinum* toxin in animal feed, which can be difficult to detect (EFSA, 2008; Liebana *et al.*, 2012).

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Brucella **spp.**

Brucellosis is a chronic and contagious infectious zoonotic disease caused by pathogens of *Brucella* spp. (Canadian Food Inspection Agency, 2011). The disease has shown to affect cattle, swine, goats, sheep, dogs, horses, caribou, reindeer, elk, coyotes, buffalo, bison, and camels, among other animals (Corbel, 2006; Adams and Moss, 2008; Canadian Food Inspection Agency, 2011; CDC, 2012). *Brucella* can affect a range of hosts of which B. abortus is typically associated with cattle, *B. melitensis* with sheep and goats, B. suis with swine, and B. canis with dogs (Corbel, 2006). Humans can contract brucellosis through exposure to infected animals. Transmission to humans can occur through environmental or occupational contact (e.g. an occupational disease of farmers, herdsmen, veterinarians, hunters, and slaughterhouse workers) or through foodborne transmission from animal products, (e.g. (raw) milk or milk products like cream or cheese or undercooked meat that are contaminated with *Brucella* spp.) (Adams and Moss, 2008; CDC, 2012). *B. melitensis* is most frequently reported as the cause of human disease (Corbel, 2006). Although considered rare, case reports of human-to-human transmission of brucellosis have been reported. A recent systematic review by Tuon *et al.* (2017) described transmission cases thus far as transplacental, from breastfeeding, intercourse, and tissues or fluids such as bone marrow and blood. Brucellosis is still very widespread in many regions of the world. In order to investigate brucellosis the WHO has recommended standards and strategies for surveillance, prevention, and control (WHO, 2015).

Literature on *Brucella* or brucellosis and the contribution of animal feed since the FAO and WHO (2008) report are not readily reported. In brief, brucellosis may spread in several ways including through direct contact with tissues or fluids of infected animals, consumption of colostrum or milk from infected animals, or consumption of feed or water that has been contaminated, e.g. from infected tissues or fluids (Canadian Food Inspection Agency, 2011).

For additional information on microbiological hazards in feedstuffs, consult Liebana *et al.* (2012), Chapter 5: Assessment of the microbiological risks in feedstuff for food-producing animals.

Brucella spp.

Hazard	*Brucella* spp.	
Source(s)	Animals: ingestion of infected tissues or fluids, such as aborted fetuses, birth tissues and fluids, and colostrum or contaminated feedstuffs. Humans: exposure to contaminated environments or objects, occupational exposure (e.g. to infected animals), foodborne (e.g. from contaminated animal products, such as (raw) milk or milk products like cream or cheese or undercooked meat).	
Transfer to food of animal origin	**Transfer:** medium	**Strength of evidence:** strong-moderate
Potential impact on human health	**Potential impact:** high	**Strength of evidence:** strong
Potential impact on animal health	**Potential impact:** high	**Strength of evidence:** strong
Knowledge gaps	Limitations in detection (including diagnostic) and prevention of *Brucella* transmission. Control and intervention methods for high-risk areas warrant further research.	

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Atypical Mycobacteria

The purpose of this section is to raise awareness of the concerns related to atypical *mycobacteria* in feed, and thus, we do not assess this information in a summary table.

Description of hazard

Mycobacteria are characterized as pleomorphic, acid-fast, non-motile, nonsporulating, Gram-positive bacteria, which are ubiquitous in water, soil, and sediment (Lewis and Chinabut, 2011; Monticini, 2010; Stoskopf, 1993). The classification of *mycobacteria* is rather complex. Atypical *mycobacteria* are classified as non-tuberculosis *mycobacteria* (NTM), environmental *mycobacteria*, or *mycobacteria* other than tuberculosis (Mott) since they do not cause tuberculosis or leprosy (Monticini, 2010).

Mycobacterium avium subspecies paratuberculosis (MAP) is a causative agent of paratuberculosis (Johne's disease) in ruminants; MAP has been reported to contaminate grass silage and as being responsible for mortality issues and economic losses. The literature on the survival of MAP in pastures, manure, and in water warrants further research (Khol, Beran, Kralik *et al.*, 2010).

Mycobacteriosis, also referred to as fish tuberculosis or fish *mycobacterium* (FishMB), is a common (sub-acute) chronic disease caused by several distinct species of Mycobacterium, the most common of which are M. marinum and M. fortuitum (Butcher and British Small Animal Veterinary Association, 1992; Lewis and Chinabut, 2011; Monticini, 2010, Noga, 1996). Given the ubiquitous nature of *mycobacteria*, mycobacteriosis continues to be documented in fish species worldwide. At least 160 species of fresh and salt-water fish have been reported as hosts for this disease; mycobacteriosis is also described in aquarium fish, which have shown higher incidences of disease - due to prolonged exposure - versus that of fish raised for commercial purposes (Lewis and Chinabut, 2011; Monticini, 2010). The prevalence of mycobacteriosis in aquarium fish and infected fish in natural populations varies from 10 to 22% and 10 to 100%, respectively (Lewis and Chinabut, 2011). *Mycobacteria* in fish continues to be a problem, and the successful vaccination of fish against *mycobacteria* is warranted. Also, rapid diagnostic kits to detect *Mycobacterium* is an urgent need for aquaculture (Lewis and Chinabut, 2011).

Sources

MAP can infect many species of domestic and wild animals worldwide. Fecal-oral transmission is the main route of infection; young animals and older ruminants are susceptible. Other transmission sources for paratuberculosis include free-range wild ruminants, contaminated pastures, and feed (Khol *et al.*, 2010). The potential survival of MAP in the environment, e.g. in pastures, manure, water and water sediments, silage, among others like feed, is relevant when considering transmission routes and the source of infection. The concern is how environmental contamination of MAP, e.g. from infected animals shedding it into the environment and its survival thereafter, as could occur with feed, contributes to oral-fecal transmission.

Mycobacteria infection in fish can occur through oral ingestion of contaminated feed, eating other infected fish, or eating the feces of other fish. Transmission can occur from open lesions, external parasites, or from bacteria that were shed from infected skins ulcers or the intestine; also transmission to eggs is possible if present in the gonads of female fish (Bassleer, 1996; Noga, 1996). *Mycobacteria* affect mostly farmed fish located in Asia and in developing countries. Open fish farming may also be contaminated by ingesting contaminated feed (such as trash fish food) and water, but contamination may also be connected to freshwater prawns (Penaeidae spp.) and other invertebrates (Lewis and Chinabut, 2011; Monticini, 2010).

Transfer to food of animal origin

Humans can become exposed to MAP through contaminated food or water.

Fish infected with mycobacteriosis, particularly fish for food, are unfit for human consumption (Lewis and Chinabut, 2011).

Potential impact on human health

MAP can cause disease in humans (Khol *et al.*, 2010).

A notable aspect of mycobacteriosis is its zoonotic transmission to humans, usually causing localized non-healing ulcers (Noga, 1996). Particularly, M. marinum has been associated with human skin problems; the so-called "swimming pool granuloma" is a hypersensitivity reaction of skin in contact with the organisms. Also, M. fortuitum has been associated with fish tank granuloma causing local abscesses in the extremities like fingers and hands (Butcher and British Small Animal Veterinary Association, 1992). This disease appears to be a low risk to human health, especially when comparing the likelihood of exposure to agents; nevertheless, caution is warranted, especially for immunosuppressed individuals (Noga, 1996).

Potential impact on animal health

MAP can have a long incubation period with clinical signs being absent in infected herds; nonetheless, infected cattle can shed MAP into the environment. As the disease is untreatable, this ultimately leads to the death of the diseased animal (Khol *et al.*, 2010).

Fish infected with *mycobacteria* can exhibit symptoms such as lethargy, hiding, decreased appetite, emaciation, exophthalmia, frayed fins, discoloration, while other signs include skeletal deformations, non-healing shallow or deep ulcers, or fin erosion (Bassleer, 1996; Butcher and British Small Animal Veterinary Association, 1992; Monticini, 2010; Noga, 1996; Stoskopf, 1993). Integrally, grey or white granulomata on internal organs of fishes especially the kidneys, liver, or spleen are clinical manifestations of *mycobacteria* (Butcher and British Small Animal Veterinary Association, 1992; Monticini, 2010; Noga, 1996; Stoskopf, 1993). Disease prevention is easier than treatment since the disease is difficult to control (Bassleer, 1996; Noga, 1996). Some control options are eliminating or effectively quarantining infected fish and disinfection (Butcher and British Small Animal Veterinary Association, 1992; Noga, 1996; Stoskopf, 1993).

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Antibiotic resistance

Description of the hazard

Antibiotic resistance is considered, by organizations like WHO, to have a major impact on treatment of pathogenic infections in both humans and animals as it limits the therapeutic options that the medical profession has at hand when the target pathogen becomes resistant. FAO has elaborated an action plan on antibiotic resistance in

Antibiotic resistance

Hazard	Antibiotic resistance	
Source(s)	Whilst there is a background presence of antibiotic resistance genes within bacterial populations in different ecosystems, the presence of antibiotics exerts a selective pressure on the preponderance of such genes within such populations. The possible role of addition of antibiotics to animal feed to resistance in intestinal bacteria (including potentially zoonotic pathogens) has not yet been completely resolved, as is the possibility that sub-inhibitory levels of antibiotics may still induce resistance. Another potential source of antibiotic resistance is the presence of resistant bacteria in feed.	
Transfer to food of animal origin	**Transfer:** Whilst pathogens in general are known to be transferred to food (section 3.2.1), there is limited direct evidence for animal feed as the starting point for a cascade of transfers of antibiotic-resistant bacterial pathogens from feed via the animal to animal-derived foods and their consumers. Indirect evidence for such a feed-food transfer also comes from data indicating transfer of antibiotic-resistant pathogens from feed to livestock animals, and from infected animals to food products	**Strength of evidence:** moderate
Potential impact on human health	**Potential impact:** failure of antibiotic treatment of infected human consumer of foods that have not been properly processed to eliminate any resistant zoonotic pathogen transferred from feed. Infection-related health syndromes are similar to those for the pathogens in general (section 3.2.1)	**Strength of evidence:** moderate
Potential impact on animal health	**Potential impact:** high, i.e. treatment of infections with antibiotics can be compromised (e.g. antibiotic resistant *Staphylococcus aureus* infecting dairy cows). Infection-related health syndromes are similar to those for the pathogens in general (section 3.2.1)	**Strength of evidence:** strong
Knowledge gaps	Impact of addition of antibiotics to feed and of sub-inhibitory levels of antibiotics on antibiotic resistance in intestinal bacteria. Occurrence of antibiotic resistant pathogenic microorganisms in feeds. Cascade of transfers of resistant pathogens via feed and animals to consumers. Impact of antibiotic-resistant pathogens transferred from feed to food as compared to impact of other infection routes for the same pathogen. Horizontal gene transfer of resistance genes present in feed, e.g. from non-viable/dead pathogens transferred via feed to food.	

food and agriculture, which follows a multi-faceted approach, including the raising of awareness, developing capacity for monitoring and surveillance, preventing infections, strengthening of governance, and promoting prudent use of antibiotics and good practices (FAO, 2016). This falls within the tripartite WHO-FAO-OIE collaboration on tackling antibiotic resistance through a "One Health" approach and thus also aligns with the WHO and OIE's action plans for human and veterinary health, respectively, focusing on awareness, knowledge, governance, international harmonization, and investment in development of new medicines (OIE, 2016; WHO, 2015).

Causes and sources

Three mechanisms of transfer of antibiotic resistance from feed to food and subsequent infection of human consumers can be discerned, in analogy with Chang, Wang, Regev-Yochay *et al.* (2015) for livestock animal – consumer transfer:

- Antibiotic-resistant bacteria that are present in feed infect livestock animals consuming this feed and are subsequently transferred to food of animal origin, from which they can infect human consumers unless adequate processing and other measures are applied to prevent such infection
- The presence of antibiotics in feed may create a selective pressure favouring the proliferation of gastro-intestinal pathogens carrying antibiotic resistance genes in livestock animals consuming this feed [e.g. (Looft, Allen, Cantarel *et al.*, 2014)]. These pathogens could then infect the animal, and be transferred to food of animal origin and infect the consumer similar to the previous bullet point.
- DNA harbouring antibiotic resistance genes could originate from these pathogens, for example as free DNA released from lysed bacteria or as part of the DNA of bacteriophages commuting between different hosts. In bacteria, various mechanisms exist for the horizontal exchange of such genes, both plasmid- and chromosome-bound, between different bacterial host species and strains.

Antibiotic-resistant bacteria in feed. A relevant item that has so far received limited attention in scientific literature is the presence of antibiotic-resistant bacteria in feed [e.g. (Martins da Costa, Oliveira, Bica *et al.*, 2007)]. Antibiotic resistance in feeds is measured, for example, as part of the characterization of some of the microorganisms, such as *Salmonella*, monitored for within national monitoring programs. In a retrospective review of 20 years of such monitoring for *Salmonella* in feed in the United Kingdom, for example, it has been observed that part of the isolates showed antibiotic resistance to a particular antibiotic, while a smaller number showed resistance against two or multiple antibiotics from different classes, including ampicillin, chloramphenicol, furazolidone, neomycin, nalidixic acid, streptomycin, sulfonamides, sulfomethoxazole/trimethoprim, and tetracycline (Papadopoulou, Carrique-Mas, Davies *et al.*, 2009). An analysis by Ge, LaFon, Carter *et al.* (2013) of vegetable and animal–derived feed ingredient samples from processing and production plants for the presence of different bacterial genera (*Salmonella*, *Campylobacter*, *Escherichia coli*, and *Enterococcus*) showed that these were indeed commonly present yet that they did not commonly harbour antibiotic resistance (i.e. with a maximum of 21% tetracycline-resistant serovars in animal-derived ingredients). Analyzing the occurrence of *Salmonella* in a wide range of feeds, Li, Bethune, Jia *et al.* (2012) found that on average 21% out of 254 isolates were resistant to at least one antibiotic. Whereas Martins da Costa, Oliveira, Bica *et al.* (2007) showed that much but not all of the antibiotic resistance present in broiler feeds corresponded to that in the ingredients from which these feeds had been produced, other reports indicate that also transfer from dust, farm workers handling feed, and infected animals coming into contact with feed are likely routes of feed contamination [e.g. (Dierikx, Van Der Goot, Smith *et al.*, 2013)].

Antibiotic resistance induced by antibiotic residues in feed. As recent metagenomic research on intestinal microorganisms in livestock has also indicated, a low background level of antibiotic resistance may already exist in animals, even in the absence of antibiotic use (Allen, 2014, Looft *et al.*, 2014). Nonetheless, the presence of antibiotics in feed consumed by livestock can induce selective pressure towards antibiotic resistance in pathogenic microorganisms occurring in the gastrointestinal tract of the animal consuming it (Looft *et al.*, 2014). This has been particularly investigated for antibiotics used historically in feed as growth promoters (i.e. deliberately added to feeds instead of adventitiously present residues), such as glycopeptides, streptogramins, macrolides, evernimicins, and bacitracin [reviewed by Wegener (2003)]. Some of the antibiotic resistance genes detected are associated with mobile genetic elements (plasmids) and can thus be transferred between the microorganisms harbouring them and other environmental recipients. This, in turn, contributes to even more widespread resistance against the particular antibiotic. Antibiotic resistance has been linked to the deliberate addition of antibiotics to feed and measures have been taken to restrict such practice [e.g. (Wegener, 2003)].

Moreover, the likelihood of resistance development in the presence of low, sublethal/inhibitory levels of antibiotics in feed has recently also started to receive attention given the induction of resistance by, for example, tetracycline, ciprofloxacin (fluoroquinolone), and streptomycin (aminoglycoside) at concentrations way below their established minimum inhibitory concentrations (Andersson and Hughes, 2014). Besides selection of resistant mutants within a population, various mechanisms may be at the cause of this phenomenon, including impacts of the antibiotic on genetic processes (horizontal gene transfer, recombination, mutation) and phenotype [metabolic arrest to sustain antibiotic exposure; (Andersson and Hughes, 2014)]. Interestingly, in their study of cross-contamination of Dutch feed mill operations producing medicated feed, Stolker, Manti, Zuidema *et al.* (2013) observed levels of antibiotics (macrolides, penicillins, sulfonamides, and tetracyclines) that were similar to those historically used for growth promotion, indicating that such levels commonly occurring might also trigger antibiotic resistance (as previously observed for antibiotics used for growth promotion; with 55% of the samples below 2.5% of the lowest registered level for the particular antibiotic, which had been proposed by Dutch policy makers as maximally acceptable carry-over). It would also be of interest to verify whether even lower levels of residues would also still be able to induce resistance. Ge, Domesle, Yang *et al.* (2017) reported the outcomes of an in vitro experiment in which *Enterococcus faecalis*, and *Enterococcus faecium* were cultured in the presence of low concentrations (0.1-0.5 µg/ml) of erythromycin, penicillin, and virginiamycin, whilst *Campylobacter coli* and *Campylobacter jejuni* were grown in the presence of erythromycin only. These concentrations were within the range of residue levels previously detected (0.1-1.5 mg/kg) in distillers grains products (dried and wet distillers grains, distillers grains solubles). Except for *E. faecalis*, the occurrence of antibiotic resistant mutants was observed. Mutants appeared to arise especially when the levels tested were 20% or more of the minimum inhibitory concentration of the antibiotic which had been observed in the particular bacterial isolate before testing started. Whilst these in-vitro findings have not been confirmed by in-vivo data, they suggest a potential hazard of stimulating bacterial resistance development in the presence of certain antibiotic residues in animal feed (Ge *et al.*, 2017).

Presence of antibiotic resistance genes amenable to horizontal gene transfer

With regard to possible indirect routes of transmission besides live resistant pathogens, Martins da Costa *et al.* (2007) caution that although microorganisms may not survive feed processing (e.g. heat treatment), genetic material from non-viable microorganisms harbouring antibiotic resistance gene may sustain these processes and hence still be available for horizontal transfer to other microorganisms. Moreover, Shousha, Awaiwanont, Sofka *et al.* (2015) observed that in chicken meat offered through retailers, bacteriophages could be isolated that were capable of infecting *Escherichia coli* and of transducing resistance to one or more of five antibiotics. Given that such bacteriophages may be more resistant towards sanitary measures than their host bacteria, they could also provide a route for horizontal gene transfer of antibiotic resistance genes to other pathogens possibly infecting the food after sanitation (Shousha *et al.*, 2015).

Transfer to food of animal origin

The transfer can be thought of as a cascade of two subsequent stages, with initially the infection of the livestock animal with antibiotic pathogens originating from the gastrointestinal tract, followed by the transfer of pathogens from the infected animal via food to the consumer. Whilst there is ample data on the transfer of antibiotic resistant pathogens in either stage providing indirect evidence that this would also hold true for the stages combined, i.e. from feed to food.

Infection of a food-producing animal with antibiotic resistant pathogens is known to have led in some cases to food infection of the consumer due to inadequate preventive measures, such as the consumption of raw, non-heated milk or other animal products [e.g. (Chang *et al.*, 2015; Oliver, Murinda and Jayarao, 2011)].

Potential impact on human health

There is a plausible link between failure of antibiotic treatment of patients sporadically infected through the consumption of food containing antibiotic-resistant bacteria, whilst this should be viewed within the context of its contribution to the overall burden of infections with antibiotic resistant pathogens (Chang *et al.*, 2015).

Potential impact on animal health

The presence of zoonotic pathogens in feed, if it occurs, is generally linked with a transmission and, in some cases, an infection risk for the livestock animal consuming it, such as for mastitis-causing *Staphylococcus aureus* from unpasteurized waste milk or colostrum from infected cows (Abb-Schwedler, Maeschli, Boss *et al.*, 2014), *Salmonella* in feed ingredients and compound feeds from contaminated feed-producing facilities, and *Listeria monocytogenes* in wet feeds and silage (Liebana and Hugas, 2012). The option to treat animal infections with antibiotics will be compromised by infections with resistant pathogens and thereby increase the impact of animal health, such as in the case of methicillin (β-lactam) -resistant *Staphylococcus aureus* infecting dairy cow udders, causing mastitis, which is commonly treated with β-lactam antibiotics (Guimaraes, Manzi, Joaquim *et al.*, 2017).

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Viruses

Crop and animal feed contamination during storage, transportation, and processing, including inappropriate heat treatments and contaminations thereafter, have been associated with additional biological hazards, including viruses. For example, the Newcastle disease (ND) can be transmitted to poultry from feral pigeons via their excreta and can contaminate improperly stored feed (Crawshaw and Fink-Gremmels, 2012). Furthermore, Crawshaw and Fink-Gremmels (2012) note other major viral diseases such as foot and mouth, swine fever, and swine vesicular disease as being transmitted to feed when animal products are incorporated into the diet of animals. Such information is widely acknowledged and regulated in practice, e.g. via HACCP; banning such waste products has helped to eradicate many diseases where this contamination route had previously been an issue. However, in the context to reduce food waste, animal products might be incorporated more frequently in feed, and consequently, the risks may increase again (see further section 4.2).

More recently, the global concerns surrounding the porcine epidemic diarrhea virus (PEDV), an alphacoronavirus that infects the small intestinal cell lining of pigs, have escalated (EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), 2014). In the following sections, PEDV will be highlighted in more detailed.

Description of hazard

PEDV is an enveloped single-stranded positive sense RNA virus from the genus Alphacoronavirus that has shown to cause severe enteric disease and high mortality in swine, especially piglets (Bowman *et al.*, 2015; Dee *et al.*, 2016; Trudeau, 2016). Both economic struggles in the swine industry as well as the burden on animal

Porcine epidemic diarrhea virus (PEDV)

Hazard	Porcine epidemic diarrhea virus (PEDV)	
Source(s)	Transmission: fecal-oral route in a herd; contaminated fomites, transport (equipment), feed storage facilities, and personnel; PEDV-positive aerosols; contaminated animal feed or ingredients	
Transfer to food of animal origin	**Transfer:** low	**Strength of evidence:** strong
Potential impact on human health	**Potential impact:** low	**Strength of evidence:** strong
Potential impact on animal health	**Potential impact:** high	**Strength of evidence:** strong
Knowledge gaps	Clinical differentiation between PEDV and transmissible gastroenteritis (Bowman, Krogwold, Price *et al.*, 2015). Lack of data on the survival of PEDV in feed and feed ingredients; the routes of entry of PEDV strains are unidentified (Dee,Neill,Singrey *et al.*, 2016). The efficacy and impact that certain intervention strategies, such as thermal or pressure processing of feed pellets and the inclusion of feed additives for anti-viral effects, have on reducing the risk of PEDV are limited (Dee, Clement, Schelkopf *et al.*, 2014; Trudeau, Verma, Urriola *et al.*, 2017). Monitoring and elimination of PEDV at animal feed manufacturing facilities (Huss, Schumacher, Cochrane *et al.*, 2017). Analytical methods: Real time-PCR detects viral nucleic acid, meaning the presence of PEDV can be confirmed, but not necessarily the presence of viable and infectious PEDV; moreover, PEDV is difficult to isolate (Bowman *et al.*, 2015; Trudeau, 2016).	

welfare, meaning the increased mortality in piglets, are shaping research efforts to control outbreaks and identify sources.

PEDV is a larger concern in Asian countries versus that of European countries because outbreaks are more acute and severe with higher mortality (Huss *et al.*, 2017). The PEDV 2013 outbreak in the USA is a recent example of where the economic losses and neonatal mortality for the pork industry are greatly emphasized (Dee *et al.*, 2016; Huss *et al.*, 2017; Trudeau *et al.*, 2017).

Sources

PEDV transmission in a herd is shown to occur through a faecal-oral route; however, other modes of transmission such as via contaminated fomites, transport (equipment), feed storage facilities, and personnel, PEDV-positive aerosols, and contaminated animal feed or ingredients have been suggested (Bowman *et al.*, 2015; Dee *et al.*, 2014; Huss *et al.*, 2017; Trudeau, 2016). In 2014, researchers identified contaminated feed, and its ingredients thereof, as a vehicle for PEDV infection in naïve pigs (Dee *et al.*, 2014). In a 2014 EFSA scientific opinion on porcine epidemic diarrhoea virus and emerging porcine deltacoronavirus, the EFSA Panel on Animal Health and Welfare summarized that PEDV transmission between farms can occur as a result of matrices contaminated with faeces such as infected animals, feed, or objects (EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), 2014).

Transfer to food of animal origin

Data are lacking on the detection of PEDV RNA or the infectious virus in pork muscle. Consumption of pork muscle tissue by humans does not appear to be of a concern as PEDV has no zoonotic capacity and no human cases have been reported (EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), 2014).

Potential impact on human health

The concern surrounding feed safety and potential epidemic transfer of porcine coronaviruses are not linked to human food safety risk, yet rather the impact on swine health and economic losses are leading factors (Trudeau, 2016).

Potential impact on animal health

In general, PEDV in swine has shown to be a highly transmissible coronavirus causing severe enteric disease in neonatal piglets. Symptoms include vomiting, anorexia, and (watery) diarrhoea, thus causing malabsorption and dehydration in swine (Trudeau, 2016; Trudeau *et al.*, 2017) as well as potential mortality rates of 80-100% (Bowman *et al.*, 2015).

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Prions

Description of the hazard

Bovine Spongiform Encephalopathy (BSE) is the main representative form of Transmissible Spongiform Encephalopathies (TSEs), caused by the presence of prions, which are modified forms of host specific proteins. The pathological form of the prion protein accumulates primarily in nervous system organs because of its resistance to proteolytic enzymes. The epidemiology and background of prions as a feed and food contaminant differs principally from those of chemical or microbiological contaminations: prion diseases always have a progressive and irreversible nature, there is a genetic basis and resistance or cure does not exist (Prusiner, 1998; Prince *et al.*, 2003; Collins *et al.*, 2004).

Prions

Hazard	Prions	
	Bovine Spongiform Encephalopathy (BSE) is the main representative form of Transmissible Spongiform Encephalopathies (TSEs), caused by the presence of prions. These proteins are strongly heat resistant, persistent in the animal system and cause irreversible neuropathological disorder. The incidence rate is increasing after the low level in 2013.	
Source(s)	Ruminant animal by-products used as feed ingredients. Some specific organs can contain prions: brain, spinal cord, trigeminal ganglia, distal ileum, spleen and eyes. These organs are indicated as Specified Risk Material (SRM) and are not allowed to enter the food processing chain (EU, USA, Canada) or are recommended to be removed (OIE).	
Transfer to food of animal origin	Transfer: High. Prions can easily be transferred when SRM is included in food of animal origin.	Strength of evidence: Strong
Potential impact on human health	Potential impact: High. Variant Creutzfeld-Jacob is the human version of BSE. vCJD, as BSE, is irreversibly lethal.	Strength of evidence: Strong
Potential impact on animal health	Potential impact: High. After a minimum level of incidences of BSE in 2013, a slight increase in EU member states occurs.	Strength of evidence: Strong
Knowledge gaps	Chronic Wasting Disease (CWD) is a TSE occurring in cervids: moose, elk, reindeer, and several species of deer primarily in North America, but recently also in Norway. Unlike BSE, feed seems not the only, not even the primary source. The causing prions can occur in a range of organs and fluids, including skin and antler velvet, muscle, saliva and blood. The contribution of feed to the spread of CWD should be further investigated. A relationship with a human version, CJD or otherwise, is investigated but still insufficiently known. Until now, only the variant version (vCJD) has been connected to BSE. There is a dispute on a putative link between BSE and sporadic (sCJD), which is the most common version of CJD.	

After the major outbreak in UK after 1988 with a maximum in 1992 (6657 incidences per million bovines), severe measures were installed in the EU to eradicate this disease. The incidence rate decreased further by more than 90% between 2007 and 2013. A slight increase is shown from 2014 for EU total (non UK) from 0 (2013) to 6 incidences per million bovines in 2015, primarily due to specific incidences, viz. Portugal and Romania in 2014 and Slovenia in 2015 (OIE, internet link 11).

A worldwide overview of the risk of BSE is presented and regularly updated by OIE (see internet link 1). It should be noted that for various countries, e.g. almost all African countries, OIE has not established an official BSE status.

Other TSEs include:

- Scrapie: found in sheep (EFSA, 2014).
- Chronic wasting disease (CWD): occurs among wild ruminants in certain areas in North America (USA and Canada), increasing in abundance (Tyler *et al.*, 2014; Haley and Hoover, 2015). CWD was encountered in Norway in reindeer and moose in 2016 (EFSA, 2017).
- Some human prion diseases appeared to be transmissible as well, especially Kuru (Collinge *et al.*, 2006) and a variant of Creutzfeldt-Jakob disease (vCJD). A relationship between BSE and vCJD has been established (Hill *et al.*, 1997; Gill *et al.*, 2013).

Sources

Elaborate analyses revealed that the most likely route for infection of susceptible animals is by oral ingestion of prions. These prions originate from certain ruminant by-products used as feed ingredients (Prince *et al.*, 2003; Morley *et al.*, 2003). Restrictions on feed vary from country to country, based on their own scientific risk assessments. The minimum ban recommended by the World Organisation for Animal Health (OIE) is on feeding ruminant by-products to ruminants with exceptions that allow for feeding e.g. milk, tallow, and gelatine (link 2; Liu *et al.*, 2011). The extent of the measures depends on the risk status of a country as evaluated by the OIE (link 3).

Several procedures developed for inactivating prions at some stage in the feed production chain, such as composting (Xu *et al.*, 2014), chlorine treatment and severe cleaning (Hawkins *et al.*, 2015), heat treatment and acid or alkaline treatment (Mekonnen *et al.*, 2013) appeared to be not fully effective. The EU system of pressure cooking (133 oC for 20 min) is not considered as a sufficient inactivation process without the supporting measures of the feed bans. Up to 2% of the prion molecules remain active (Oberthür, 2004). Only severe treatments (180°C for 3 h) resulted in full inactivation (Yoshioka *et al.*, 2013).

Transfer to food of animal origin

Prions act as triggers which stimulates indigenous proteins to get folded in a wrong, persistent shape. The possibility to have different three dimensional forms for the relevant protein depends on nucleotide mutations in the coding gene sequence. Taking the situation of BSE, this process of misfolding and accumulation occurs in a set of specific organs: brain, spinal cord, trigeminal ganglia, distal ileum, spleen and eyes, together indicated as Specified Risk Material (SRM; Pitardi *et al.*, 2013). The brain and spinal cord count for 90% of the present prions, if any. BSE prions are so far not found in any other organs (Morley *et al.*, 2003). If SRM can enter the food production chain, the probability of transfer of prions exists. The presence of SRM in food of animal origin is prevented, in some cases depending on the age of the animal.

Potential impact on human health

Among different versions of Creutzfeld-Jacob disease, an irreversible and fatal neuropathological disorder, a variant version (vCJD) related to BSE has been established (Gill *et al.*, 2013).

A severe outbreak of vCJD did not occur until now. Until 2013 a total of 177 vCJD cases have been reported for the United Kingdom, and a total of 226 worldwide. No incidences have been reported for 2014 (link 4). This occurrence has been related to the BSE epidemic in the late 1980s and early 1990s, which points to an incubation time of approx. 13 years with a 95% confidential interval of: 9,7-17,9 years (Chadeau-Hyam *et al.*, 2010). Even longer incubation times have been considered for Kuru, up to 56 years (Collinge *et al.*, 2006). Such long incubation times have been suggested for vCJD as well, which has the consequence that a (second) outbreak of vCJD can occur in the future. Above that, the distribution of the current cases of vCJD shows uncertainties in the future predictions (Garske and Ghani, 2010). Possible human-to-human haematogenous transmission might complicate the evaluation of the occurrence of vCJD (Collins *et al.*, 2004).

Potential impact on animal health

In general, BSE in cattle shows neurological signs and deteriorating behaviour, such as paralysis, disorientation and lethargy. In addition, drop in milk production, anorexia and an increase in aggression is noted. A Decision Support System for clinical diagnosing BSE in cattle is available (Saegerman *et al.*, 2004). The disease is progressive and will result in the death of the animal in all cases.

Knowledge gaps

Chronic wasting disease (CWD) is a prion disease related to BSE. The incidences of CWD in Northern America are increasing and spreading (Haley and Hoover, 2015). CWD was encountered in Korea (by means of imported deer from Canada, early 2000). In Norway a total of five incidences of CWD were found in moose and reindeer during 2016 (VKM, 2017). An extensive literature overview, current status and risk assessment has been carried out by EFSA, also pertaining to regions outside Europe (EFSA, 2017).

There is a range of routes for infection of cervids. Vectors for transmission include urine, faeces, saliva, nasal secretions, milk, semen and antler velvet. Blood and meat are shown to be capable of containing prions, in contrast to BSE. Plants such as alfalfa, corn and tomatoes can take up the prions causing CWD, and parts of these plants can infect mice after injection (internet links 5 and 6; Pritzkow *et al.*, 2015). Prions remain infectious for long periods in the environment (see abstracts AD. 81-83 of internet link 7). Although the most plausible route seems to be oral exposure, feed is not considered the primary source. This conclusion is influenced by the situation that for CWD primarily wild animals are involved. Artificially produced compound feed is not at stake in the situation of wild animals. Here, plants that can harbour the prions from the environment are the replacement of "feed". Additional feeding for wild animals under specific circumstances, and feed in general for animals in captivity, are certainly an issue. This includes fodder and roughage. All types of feeding can be sources included in the oral route. In addition, maternal transmission is encountered experimentally. With this variety of transmission routes, CWD is highly contagious, unprecedented among TSEs.

Squirrel monkeys appeared to be able to be infected by CWD prions at a rate of 15% after oral exposure (Race *et al.*, 2009; referred in EFSA study). This primate species is not closely related to humans. CWD prion strains are capable of adaption to specific interspecies barriers. Infection of several in vitro and in vivo human model systems with CWD prions resulted in PrPSc strains resembling the version of sporadic CJD (sCJD; Barria *et al.*, 2011, 2014; Davenport *et al.*, 2015, referenced in EFSA study). Summarising, the evidence for a link between human TSEs (indicated generally, apparently including both sCJD and vCJD) and CWD is considered weak but not absent (as of December 2016, date of closure of the EFSA study).

In 2017 some new data and an overview has been published. Waddel *et al.* (2017) carried out a meta-study of 23 principal reports on CWD transmissibility out of a set of 800 citations. Evidence was collected in the areas of epidemiology, in vitro and in vivo studies. In total 14 studies (5 epidemiological, 2 with macaque primates, 7 with humanized mice) revealed no transmission of CWD agents. The other nine studies (2 with squirrel monkeys, 7 in vitro studies) showed either infection or at least misfolding of the relevant proteins caused by CWD agents. Waddel *et al.* (2017) consider this information as "prudent" for the future, since incubation time of CWD can be as long as decades. A long running experiment with macaque primates is carried out by a team originating from five institutes in two countries (Canada, Germany). Infection routes of CWD containing material include intracerebral, intra-gastric, oral routes and blood transfusion. In 2015 no clinical nor neuropathological signs were found, although earlier studies showed transmissibility of BSE and classical scrapie to macaques (Mussil *et al.*, 2015, included in the EFSA study). In the framework of the same international study, in May 2017 during the Prion Conference neuropathological signs were reported for four out of ten macaque primates (Czub *et al.*, 2017: link 8). The experiment will continue until 2018. A recent study showed that new emergent strains of CWD prions can contribute to the expansion of the host range (Herbst *et al.*, 2017).

Principally the variant version of CJD is linked to the prions causing BSE (Hill *et al.*, 1997; Gill *et al.*, 2013). The relation between BSE and sCJD is disputed (Mead *et al.*, 2000; Schoch *et al.*, 2006). Until now, the US Center for Disease Control (CDC) has reported and documented four cases of vCJD among American citizens, of which three most likely got infected outside the USA (link 9). It is also reported that the number of cases of CJD in general is increasing over the years in the USA, from approx. 250 at the start of the millennium to 481 in 2015. These numbers apply to sporadic CJD in most cases. Aging of the US population and better surveillance are considered the most important factors. The simultaneous rise of CWD cases among cervids gave nevertheless rise to concerns (Haley and Hoover, 2015; link 10). In the light of a putative relationship between BSE and sporadic CJD, insufficient knowledge of the mechanisms of the CWD prions, and the long incubation time of CWD, further research is needed.

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Internet links (last accessed on 1st July 2019)

1. http://www.oie.int/en/animal-health-in-the-world/official-disease-status/bse/list-of-bse-risk-status/
2. http://www.oie.int/fileadmin/Home/eng/Health_standards/tahc/current/chapitre_bse.pdf
3. http://www.oie.int/en/animal-health-in-the-world/official-disease-status/official-recognition-policy-and-procedures/
4. https://ecdc.europa.eu/en/vcjd/facts
5. http://wisconsinwatch.org/2013/09/prions-in-plants-new-concern-for-chronic-wasting-disease/
6. http://www.abstractsonline.com/Plan/ViewAbstract.aspx?sKey=cf0f185d-6f87-40f8-af8c-19d631e62561&cKey=219cd4d8-9980-4835-82cb-7649156010fa&mKey={40C89FC9-A586-491D-A3C7-B0F26504839B}
7. http://www.tandfonline.com/doi/pdf/10.4161/pri.24864
8. http://chronic-wasting-disease.blogspot.nl/2017/06/prion-2017-conference-abstract-first.html
9. https://www.cdc.gov/prions/vcjd/vcjd-reported.html
10. http://www.jsonline.com/story/news/local/wisconsin/2017/07/07/can-chronic-wasting-disease-jump-humans-concerns-keep-rising/453371001/
11. http://www.oie.int/animal-health-in-the-world/bse-specific-data/annual-incidence-rate/

Endoparasites

Description of hazards

Endoparasites are parasites that live in the body of the host. Some endoparasites such as *Taenia*, *Diphyllobothrium*, *Echinococcus*, *Trichinella* and *Toxoplasma* are of a human health concern and can be associated with animal feed.

In the phylum Platyhelminthes, an important class of Cestoda includes the tapeworms of the genera *Taenia*, *Diphyllobothrium*, and *Echinococcus*. *Taenia*sis is the intestinal infection of tapeworms, of which *Taenia solium* (pork tapeworm) and *Taenia saginata* (beef tapeworm) are the most important. *Cysticercosis* is the infection of tissues caused by cysticerci resulting from ingestion of *Taenia* eggs (CDC, 2010; WHO, 2014). Diphyllobothriasis is the infection caused by tapeworms from the *Diphyllobothrium* genus of which is commonly caused by *Diphyllobothrium latum. Echinococcosis* is a parasitic disease of tapeworms from the *Echinococcus* genus and is a zoonosis (Eiras, 2008).

In the phylum Nematoda, *Trichinella* is a related genus. Trichinosis is caused by nematodes (roundworms) from *Trichinella* spp. and is acquired by ingesting meat containing cysts (encysted larvae). *T. spiralis* is the classical causative agent (Adams and Moss, 2008; CDC, 2012).

In the phylum Apicomplexa, which contains parasitic protozoa propagated by spores, *Toxoplasma* is a related genus. Toxoplasmosis is caused by *T. gondii*, which mainly infects warm-blooded animals, including humans (Adams and Moss, 2008; CDC, 2015).

Endoparasites

Hazard(s)	Endoparasites	
	Taenia, including *T. solium and T. saginata* and eggs (causing cysticercosis) *Diphyllobothrium*, including *D. latum* *Echinococcus* *Trichinella*, including *T. spiralis* *Toxoplasma*, including *T. gondii*	
Source(s)	*Taenia*: Ingesting eggs (larve) coming from undercooked meat such as pork or beef. *Diphyllobothrium*: Consumption of infected fish. *Echinococcus*: Ingesting eggs from contaminated food, water, or soil, or by direct contact with animal hosts. *Trichinella*: Consumption of infected raw or poorly cooked meat; horse and game meat are reported as secondary food vehicles. *T. nativia* has been noted to occur in the meat of carnivores such as polar bears and walruses. *Toxoplasma*: Consumption of raw or undercooked meat, especially pork or mutton, yet game meat (red meat and organs) have also been reported. Fresh produce, seafood, and dairy products have been reported as secondary food vehicles.	
Transfer to food of animal origin	**Potential Transfer:** medium	**Strength of evidence:** weak
Potential impact on human health	**Potential Impact:** high	**Strength of evidence:** strong
Potential impact on animal health	**Potential Impact:** high	**Strength of evidence:** strong
Knowledge gaps	A clear transmission resulting directly from contaminated animal feed is less readily documented.	

Legend: Impact / Transfer can be high / medium / low; Strength of evidence can be strong / moderate / weak

Sources

During a parasites life cycle, often the eggs (larvae) or cysts (or oocysts) are ingested by a suitable host.

For *Taenia*, humans can become infected by ingesting eggs (e.g. from fecally contaminated food such as undercooked meat) (CDC, 2010, WHO, 2014). For *Diphyllobothrium*, D. latum is cestode that affects freshwater fish (Woo, 2006) and infections in humans are linked to the ingestion of infected fish (Eiras, 2008). For *Echinococcus*, echinococcosis in humans is caused by larval stages of cestodes. Humans can also become infected by ingesting these eggs from contaminated food, water, or soil, or by direct contact with animal hosts (CDC, 2012; WHO, 2014).

For *Trichinella*, several animal hosts can become infected; in humans, this mainly occurs upon consumption of infected raw or poorly cooked meat (Adams and Moss, 2008; CDC, 2012). Besides *Trichinella* spiralis other *Trichinella* spp. are less commonly reported as the cause of human disease, yet may be found worldwide from infected wild animals (CDC, 2012). For example, horse and game meat have been reported as secondary food vehicles (FAO/WHO, 2014). Also, *T. nativia* has been noted to occur in the meat of carnivores such as polar bears and walruses (Adams and Moss 2008).

For *Toxoplasma* gondii, several routes can cause infection in humans including the consumption of raw or undercooked meat, especially pork or mutton, yet game meat (red meat and organs) have also been reported (Adams and Moss, 2008; FAO/WHO, 2014; CDC, 2015). Fresh produce, seafood, and dairy products have been reported as secondary food vehicles (FAO/WHO, 2014).

Transfer to food of animal origin

A multi-criteria based ranking for risk management of food-borne parasites FAO/WHO (2014) has globally ranked several food-borne parasites by importance including an indication to their primary food vehicle as well as specific considerations for risk management at several points along the food chain. The top four (of 24) parasites and food vehicles were noted as *Taenia solium* – pork, *Echinococcus granulosus* – fresh produce, *Echinococcus multilocularis* – fresh produce, and *Toxoplasma gondii* – meat from small ruminants, pork, beef, game (red meat and organs). *Trichinella spiralis* – pork, *Trichinella* spp. - game meat (wild boar, crocodile, bear, walrus, etc.), *Taenia saginata* – beef, and Diphyllobothriidae – fresh and salt-water fish were ranked at 7th 16th, 19th, and 23rd, respectively. Nevertheless, a clear transmission resulting directly from contaminated animal feed is less readily documented. Overall, it is important to realize that endoparasites can be a hazard in feed despite the limited evidence to date. The transfer of endoparasites to feed is more likely to be associated with particular types of feed e.g., pasture feed, aquaculture, or open feeding systems that allow for contact with wildlife, versus that of a control production environment.

Potential impact on human health

As outlined in the reports from FAO and WHO (2008), endoparasites including *Taenia solium* (*Taenia*sis or *Cysticercosis*), *Echinococcus* (echinococcosis), *Toxoplasma* gondii (toxoplasmosis) and *Trichinella* (trichinellosis or trichinosis) present a risk to human health and may inadvertently contaminate animal feed (FAO/WHO, 2014). Furthermore, experts from the Joint FAO/WHO Meeting on hazards associated with animal feed (held in May 2015), indicated that D. latum (causing diphyllobothriasis) is an additional hazard of concern since human infections caused by this cestode have been repeatedly reported and linked to the ingestion of infected fish. It has also been hypothesized and suggested that the risk of a similar anaphylactic reaction in humans from larval tapeworms can occur (Vázquez-López, de Armas-Serra *et al.*, 2001; Vázquez-López, De Armas-Serra *et al.*, 2002; Eiras, 2008).

For additional information on risk management strategies for the abovementioned parasites, consult the FAO/WHO (2014) 'Multicriteria-based ranking for risk management of food-borne parasites.'

Potential impact on animal health

For *Taenia*, pigs or cattle become infected with *T. solium* or *T. saginata*, respectively, by ingesting vegetation contaminated with the eggs or gravid proglottids (i.e. a segment with eggs inside) (CDC, 2010; WHO, 2014). For *Diphyllobothrium*, first intermediate hosts include crustaceans, such as copepods, while second intermediate hosts include freshwater, anadromous, and marine fish. Definitive hosts include carnivore mammals, fish-eating birds, and humans (Wittner, White Jr *et al.*, 2011; Geerts, 2015; Diemert, Powderly *et al.*, 2017). D. latum affects freshwater fish such as pike or walleye (Stoskopf 1993); humans are the primary definitive host (Geerts, 2015; Diemert, Powderly *et al.*, 2017). For *Echinococcus*, after intermediate hosts, such as sheep, goats, swine, cattle, among others, ingest the eggs, these eggs hatch and continue the cycle (CDC, 2012; WHO, 2014). Some larval tapeworms have shown to induce anaphylactic reactions in animals fed fish meat infected by with larvae (Vázquez-López, de Armas-Serra *et al.*, 2001, Vázquez-López, De Armas-Serra *et al.*, 2002; Eiras, 2008).

For *Trichinella*, several animal hosts can become infected such as pigs, horses, wild animals (including game), and carnivorous animals like polar bears and walruses (Adams and Moss, 2008) (CDC, 2012).

For *Toxoplasma* gondii, definite hosts include domestic or wild cats (Adams and Moss, 2008; CDC, 2015).

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PHYSICAL HAZARDS

Hardware disease, also known as traumatic reticulo-peritonitis, is the term used when complications occur after an animal consumes metallic objects such as nails, wires, needles, or even tin cans. This condition is particularly associated with bovine as they do not use their lips to discriminate between materials and they do not completely chew their feed before swallowing. The majority of affected cattle are dairy and are older than two years of age. Cattle are inquisitive and tend to consume all sorts of objects while foraging. But small ruminants are selective feeders and ingest significantly less amount of foreign bodies compared to cattle (Anteneh and Ramswamy, 2015; van Raamsdonk *et al.*, 2011). The typical foreign body often encountered is a metallic object, such as a piece of wire or nail, usually greater than 2.5cm in length. Heavy foreign materials (nails, wires) may remain in the reticulum for the life of the cow. If the foreign objects puncture the heart, which is in close proximity to the reticulum, sudden death occurs (Anteneh and Ramswamy, 2015). Hardware disease is not limited to cattle and it is not restricted to animals simply grazing. Commercial feed producers go to great lengths to keep foreign materials out of their products but objects have been known to slip in unknowingly including metallic materials. When hay is cut and baled, debris in hay fields can also be trapped in the bales and innocently fed to cattle. Once these penetrating sharp foreign bodies of the needle and fish hook type enter the animal, they may penetrate any part of the digestive tract. These may be the mouth, the esophagus, the pharynx, the stomach, the intestine, the diaphragm, the pericardium or any visceral organs, which can lead to death of the animal if not intervened (Anteneh and Ramswamy, 2015).

Cattle, especially young ones, are curious and like to chew on anything within reach. They may eat large pieces of plastic (e.g. bags), baling twines, net wraps or other debris that end up in their pasture or pen or are included in forage. Consumption by cattle of diets containing plastic debris can lead to a syndrome known as software disease (Thomas, 2016). Signs of the syndrome are loss of appetite, diarrhea and excessive thirst. If the accumulation of plastic is excessive, it can result in wasting, digestive tract blockage and death. There is no way to remove the blockage without surgery. Without knowing the problem, few veterinarians will perform exploratory surgery on cattle (Thomas, 2016). In beef cattle that were fed forage that was chopped without removing the indigestible plastic net wrap, a large proportion of the net wrap was retained within the digestive tract. The material was retained almost exclusively in the rostral (reticulum and rumen) region as large, ball-like

Physical hazards

Hazard	Large metallic and plastic objects	
Source(s)	Anthropogenic: nails, wires, needles, tin cans, plastic bags, baling twines, net wraps	
Transfer to food of animal origin	**Transfer:** negligible	**Strength of evidence:** strong
Potential impact on human health	**Potential impact:** not applicable	**Strength of evidence:** not applicable
Potential impact on animal health	**Potential impact:** high	**Strength of evidence:** strong
Knowledge gaps	-	

masses (Pizol *et al.*, 2017). The authors advise that forage containing plastic net wrap should not be offered for extended periods of time due to the risk of developing software disease (Pizol *et al.*, 2017).

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Hazards of feed and products of feed production technologies of increasing relevance

In this chapter hazards of several types of feed ingredients and products of feed production technologies of increasing relevance are described. Feed and feed ingredients of increasing relevance include insects and their products (e.g. insect proteins; section 4.1), former food products and food processing by-products (section 4.2), biofuel-by-products (section 4.3), aquatic products of animal origin (fish hydrolysates and krill; section 4.4) and aquatic products of plant origin (algae; section 4.5). Products derived from production technologies of increasing relevance include products obtained by means of genetic modification and synthetic biology (section 4.6) and products obtained through nanotechnology (e.g. feed additives in the form of nanoparticles; section 4.7).

Besides the products described in this chapter other products are also used more frequently. One example is the use of industrial by-products as feed ingredients. Many minerals and trace elements now are recouped from other industries, e.g. lime from kiln dust, copper reclaimed from circuit boards, batteries and hazardous waste sites. While the materials are often of an industrial grade, the recovery of minerals and trace elements can lead to hazardous residues of heavy metals, dioxins, PCBs and other processing chemicals. Dioxins can also be formed through the recovery process, viz. heating during recycling of zinc-oxide (see section 3.1.1.1).

INSECTS

Description of the feed products

The group of insects contains many different species, with more than 1900 edible species (Van Huis, 2013). Insects are used traditionally as a protein source for animals. In the future, insects are expected to gain more and more interest as an alternative protein source, produced commercially and in large amounts for feed production. Insects may be able to partially replace traditional feed sources such as soy, maize, grains and fishmeal.

The most important insects produced for feed and food are crickets, mealworms, flies and silkworms (EFSA, 2015). Insects with the largest immediate potential for large-scale feed production are larvae of Hermetia illuscens (black soldier fly), Musca domestica (common housefly) and Tenebrio molitor (yellow mealworm). Producers in China, South Africa, Spain and the United States are already rearing large quantities of flies for aquaculture and poultry feed by bioconverting organic waste (FAO, 2013).

Insects

Product	Insects and insect-derived materials, used as feed ingredients; marketed as (i) whole insects; (ii) processed into e.g. a powder or paste; (iii) as an extract such as a protein isolate or fat/oil. *Note:* Insects consist of a very wide and diverse group of species
Potential hazards	• General: The presence / level of potential hazards strongly depends on the substrates used, the insect species, the harvest stage, the farming and harvesting conditions and post-harvest processing • Biological: pathogenic bacteria, viruses, prions • Chemical: chemical contaminants, such as heavy metals, dioxins, veterinary drug residues, pesticide residues, mycotoxins, plant toxins, insect toxins - The limited data available indicate that insects may accumulate heavy metals, in particular cadmium, from their substrates; accumulation of mycotoxins seems unlikely. For the other chemicals too few data are available to draw conclusions. • Other: allergic proteins
Potential human and animal health impact & feed-animal transfer	Due to lack of scientific data, it is difficult to fully evaluate the potential impact on human and animal health at this time.
Knowledge gaps	**Hazards** • Pathogens: Scientific studies on the occurrence of human and animal bacterial pathogens in insects processed for feed are very scarce (EFSA, 2015). • Contaminants: Data on transfer of chemical contaminants from different substrates to insects are very limited (EFSA, 2015). • Pathogens and other hazards: The risk related to the use of manure and sewage sludge as substrates should be specifically evaluated, taking into account the kind of treatment applied (EFSA, 2015). • Allergens: It is advisable that food-producing animals fed on insect proteins are monitored for allergic reactions, in order to gain more insight in the relevance of potential allergenicity for animals (EFSA, 2015) and transfer of allergenic peptides to edible animal products after their uptake from feed. **Baseline consumption data** • There is a lack of information related to the magnitude and frequency of feeding of insects and derived products to farm animals (EFSA, 2015).

In general, there are three different ways of using insects for animal feed: as whole insects; processed into e.g. a powder or paste; and as an extract such as a protein isolate, or fat/oil. Whole insects are processed (e.g. blanching, chilling and drying) with the aim of extending shelf life and also reducing microbial load. Insect meal/paste is obtained by milling either after drying (powder) or direct milling (paste) (EFSA, 2015).

In feed production insect derived products rather than whole insects will increasingly be used; insect protein, insect meal and fat fractions may be used as feed ingredients. Different insect species contain different protein levels, and the amino acid composition also varies widely. A variety of feeds (substrates) for rearing insects can potentially be used, such as compound feed (e.g. chicken feed or feed manufactured specifically for insects), grains, vegetables, supermarket returns, by-products from slaughter houses, kitchen waste, gardening and forest waste, manure, intestinal content and sewage sludge (EFSA, 2015). Due to the decreasing availability of fish oil and marine proteins, the aquaculture industry has started to move towards more vegetable ingredients in fish diets. Henry *et al.* (2015) reviewed the suitability of various insect species in the diet of farmed fish. They concluded that insects offer a good alternative to traditional fishmeal, particularly if used in a mixture of protein sources or supplemented with other ingredients.

Potential hazards

Recently, some review and opinion papers have been published on the microbiological and chemical safety of insects and their derived products used for feed and food (EFSA, 2015; Belluco *et al.*, 2013; Van der Spiegel *et al.*, 2013; van Raamsdonk *et al.* 2017; NVWA, 2014; FASFC, 2014; Rumpold and Schlüter 2016; Sánchez-Muros, 2014; Makkar *et al.*, 2014; Feng *et al.*, 2017). EFSA (2015) concluded that for both biological and chemical hazards, the specific production methods, the substrate used, the stage of harvest, the insect species and developmental stage, as well as the methods for harvesting and further processing will all have an impact on the occurrence and levels of biological and chemical contaminants in food and feed products derived from insects. Moreover, EFSA (2015) concluded that studies on the occurrence of human and animal bacterial pathogens in insects processed for food and feed are very scarce in the scientific literature and that data on transfer of chemical contaminants from different substrates to insects are very limited.

Chemical and microbiological hazards can be introduced or formed in concentrations that may be harmful to animal and/or human health. The substrate used for insect growing and the housing conditions are very relevant in this respect, and should follow good agricultural practices, like with production animals (e.g. chicken, pigs). Substrates used for insect rearing may be contaminated with mycotoxins, plant toxins, dioxins, heavy metals, veterinary residues (including antibiotics), pesticides, pathogens, viruses, parasites and prions (EFSA, 2015). During rearing, insects may be able to convert or accumulate contaminants present in their substrate, which can result in degradation or increase of the concentration of chemicals (Van der Spiegel *et al.* 2013). In this regard, safe levels for chemical contaminants in feed for production animals may not be safe when it is used as substrate for insects.

It is clear that insects (as mechanical vectors) are able to transmit pathogens to farm animals, but they may also become internally contaminated. For instance, feeding experiments of Musca domestica with substrate contaminated with E. coli O157:H7 showed that the ingested pathogens were harboured in the intestine of the houseflies, and were excreted for at least 3 days after feeding (Kobayashi *et al.*, 1999). However, according to a recent review, the main aspect impacting microbiological contamination is not the microflora of the live insect, but the safe processing and storage of derived (feed) products (Belluco *et al.*, 2013). In this respect, hygienic handling and correct storage conditions should be strongly addressed (Klunder *et al.*, 2012). Pathogenic bacteria (such as *Salmonella* spp., Campylobacter spp. and verotoxigenic E. coli) may be present in non-processed insects depending on the substrate used and the rearing conditions. Most likely the prevalence of some of these pathogens, for example Campylobacter, will be lower compared to other non-processed sources of animal protein, since active replication of the pathogens in the intestinal tract does not seem to occur in insects. Furthermore, the risk of transmission of these bacteria could be mitigated through effective processing (EFSA, 2015). The risk related to the use of manure and sewage sludge as substrates should be specifically evaluated, taking into account the kind of treatment applied, which can minimize, as in the case of treatment with high temperatures, the microbial contamination. In this case, the possible presence of spore-forming bacteria, which can survive heat treatment, must be carefully considered (EFSA, 2015). It was shown that *Salmonella* spp. can also be reduced in a system where manure is composted by black soldier fly larvae (Lalander *et al.*, 2015).

Insect pathogenic viruses occurring in insects produced for food and feed are specific for insects and therefore are not regarded as a hazard for vertebrate animals and humans. The current collective evidence shows that viruses of vertebrates can survive in substrates and be picked up by insects produced for feed via the substrate. The key issue here is the risk of transmission. This risk could be mitigated through proper choice of substrate and effective processing (EFSA, 2015).

According to EFSA (2015), data on transfer of chemical contaminants from different substrates to insects are very limited. Several chemicals may accumulate, but data are lacking to conclude on the extent of accumulation (EFSA, 2015). Charlton *et al.* (2015) investigated the possible presence of a wide range of chemical contaminants - amongst others residues of veterinary drugs and pesticides, mycotoxins, heavy metals, and dioxins - in larvae of four different fly species, being the house fly (Musca domestica), blue bottle (Calliphora vomitoria), blow fly (Chrysomya spp.) and black soldier fly (Hermetia illucens). The larvae were produced in different geographical locations, with diverse rearing methods, using different waste materials. Levels of the contaminants in the larvae were below recommended maximum concentrations (as suggested by bodies such as the World Health Organization, Codex and the European Commission). However, the heavy metal cadmium was found to be above the European Commission limit for cadmium in animal feed in three of the M. domestica samples analysed (Charlton *et al.*, 2015).

Recently, some other studies into the accumulation of chemical contaminants from substrates to insects have been performed, for heavy metals (Diener *et al.*, van der Fels-Klerx *et al.*, 2016) and for the mycotoxins DON (Van Broekhoven *et al.* 2017) and aflatoxin B1 (Bosch *et al.*, 2017). Diener *et al.* (2015) found a bio-accumulation factor of cadmium in black soldier fly larvae of 2.3-2.9 in the prepupae phase. The adult insect had, however, much lower cadmium concentrations. For zinc and lead, the bio-accumulation was below 1 in both phases. Van der Fels-Klerx *et al.* (2016) showed clear difference in bio-accumulation of three different heavy metals in larvae from black soldier flies and from mealworms.

Lead and cadmium accumulated in the black soldier fly larvae, whereas arsenic accumulated in the mealworms. Van Broekhoven *et al.* (2017) and Bosch *et al.* (2017) investigated two different mycotoxins, and found no accumulation of, respectively, deoxynivalenol in mealworm larvae, and aflatoxin B1 in larvae of both mealworms and black soldier fly. These studies prove that accumulation from substrate to insect can occur and depends amongst others on insect type, harvest stage and type of contaminant. Hence, more such studies are needed to obtain a full overview of the excretion or accumulation of chemicals from substrates.

Although prions cannot be expressed in genomes of insects (Thackray *et al.*, 2012), they may act as mechanical vectors in case of infected substrates (Post *et al.*, 1999; Van Raamsdonk *et al.*, 2017; EFSA, 2015). As such, the use of substrates of non-ruminant origin should not pose any additional risk compared to the use of other food or feed, while the risk posed by the use of substrates based on ruminant animal by-products and human waste should be specifically evaluated (van Raamsdonk *et al.*, 2017; EFSA, 2015).

No information of allergic reactions caused by consumption of insect-derived feed are reported in farm animals in the literature. It is advisable that food-producing animals fed on insect proteins are monitored for allergic reactions, in order to gain more insight in the relevance of potential allergenicity for animals (EFSA, 2015).

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FORMER FOOD PRODUCTS AND FOOD PROCESSING BY-PRODUCTS

Description of the feed products

It is evident that during the whole food supply chain (FSC), part of the biomass harvested is not intended and cannot be used for human consumption. Most of the ingredients of animal compound feeds or of other processed (wet) feeds, in this report

Former food products (FFP)

Product	Former food products (FFP):
	• Food products that passed the expiry date, products with mislabelling, packaging damage, etc. • Kitchen and catering waste **Food processing by-products:** • By-products of food processing, e.g. dairy, vegetables and fruits • By-products of food production technologies of increasing relevance *NOTE* 1: Food processing by-products such as cereal by-products (e.g. bran), corn by-products (e.g. corn gluten feed) and oilseed by-products (expellers and meal), which have a long history of application in feeds are not covered in this section. Hazards of these regular by-products are covered in Chapter 3. *NOTE* 2: Some food processing by-products are described separately in other sections of Chapter 4, viz. hydrolysates and silage from fish by-products (section 4.4) and products derived from algae (section 4.5).
Potential hazards	**Biological:** - Classical swine fever, through the feeding of kitchen waste (swill) • Foot and mouth disease: Bone marrow is indicated as carrier of the virus. Among a range of other factors, non-deboned meat and swill can act as vector for transmission. **Chemical:** • Specific for former food products: - Plasticisers or dispersants as part of packaging material, which can diffuse into the packed food material. Phthalates are the most common group. - Certain raw materials for plastic production are classified as endocrine disruptors (e.g. bisphenol A). - Some printing inks (isopropylthioxanthone (ITX)) show toxic properties. - Acrylamide and semicarbazide in bakery waste **Other hazards:** • See Chapter 3 for further reference to chemical hazards. **Physical:** • Remnants of packaging materials in former food products, e.g. plastic, metal, aluminium and glass in bakery products.
Potential human and animal health impact & feed-to-animal-product transfer	**Biological:** • Viral diseases, such as classical swine fever and foot and mouth disease are a considerable hazard for farmed animals and may lead to death **Chemical:** • See chapter 3 for hazards already covered, such as dioxins, potentially toxic elements and mycotoxins **Physical:** • Remnants of packaging materials in former food products: hazard for animals depends on the particle size and shape: a few large particles can result in obstipation, and sharp edges can result in intestine damage
Knowledge gaps	• Data on the global and regional application (types and volumes) of various former food products and food processing by-products of increasing relevance as feed materials. • The most prominent and relevant hazards of former food products and food processing by-products of increasing relevance have to be inventoried, worldwide as well as regionally. • With respect to viral diseases, the impact of feed (swill, etc.) as transmission vector should be investigated, including the contribution of animal proteins. • Chemical compounds, originating from packaging material from former food products, such as phthalates, endocrine disruptors, colorants and printing inks, need to be monitored for their presence in the chain former food product -> feed material -> animal product. • Physical hazards are largely overlooked. Occurrence, abundance and risks of remnants of packaging material need to be investigated.

referred to as food-processing by-products, are by-products of the FSC. Plant material and chaffs (cereal processing), stems, leaves, peelings, scrapings and pulp (tuber and root processing), expellers, hulls, meal and proteins (vegetal oil production), fruit pulp (wine and juice processing), brewers' grain and mash filter grains (beer and spirit production) are all recognised feed ingredients, in use for decades or centuries. The potential hazards associated with these food-processing by-products are described in Chapter 3.

Next to these established by-products, other food-processing by-products are becoming of increasing relevance. This refers among others to the processing of dairy, vegetables and fruits and the by-products of food production technologies of increasing relevance. Food-processing by-products of increasing relevance may comprise among others sugar cane tops and fruit and vegetable waste from industrial processing, including tomato pulp and pomace (FAO, 2016). Peels, rinds, pomace, pulp, culls, or other similar material generated from processing fruits or vegetables for human consumption is also described by FDA as one of the categories of food-processing by-products (FDA, 2016). It should be noted that the borderline between established and "novel" by-products is difficult to draw because of regional differences. A by-product that has an established use in one part of the world may be of increasing relevance in another part and vice versa.

Former food products (FFP) comprise among others food products that passed the expiry date, products with mislabelling, packaging damage, etc. and kitchen and catering waste. Until now a definition of FFP that is adopted or accepted by international bodies is lacking. Previously FAO (2011) applied the words "waste and losses" when referring to "the masses of food lost or wasted in the part of food chains leading to edible products going to human consumption". Therefore food that was originally meant for human consumption is considered as food loss or waste even if it is then directed to feed. Recently, the FAO Global Initiative on Food Loss and Waste Reduction (Save Food) Working Group recommended that FAO should revise the livestock related definitions in the 'Definitional framework of food loss' either by removing them or by reflecting the use of foodstuffs for feed as conversions and value addition rather than as food loss and waste (FAO, 2016). In this context it is also important to note that in the food waste hierarchy, the recycling of food waste into animal feed is regarded as the third best option with only prevention and re-use as food as better options (Papargyropoulou *et al.*, 2014). For that reason and also reflecting the preferred option of the experts involved in the Joint FAO/WHO Expert Meeting on Hazards associated with animal feed (May 2015), the wording "former food products" is used in this background document.

The total amount of former food products (FFPs) or edible by-products resulting from the FSC was estimated to be 1.3 Gtonnes in 2007 (22% of a total production of over 6 Gtonnes) (FAO, 2013). Substituted to the several steps in the FSC, agricultural production (28-36%) and consumption (3-38%) are the major sources, depending on the geographic region. The most important commodities are cereals (0.3 Gtonnes, 14% edible waste) and vegetables (0.27 Gtonnes, 30% edible waste) (FAO, 2013).

Product types with high moisture, such as dairy products and fruits, are usually intended for fermentation. As an exception, whey powder is a by-product with an established use in feed. The use of meat by-products or of FFPs containing animal products (García *et al.*, 2005) as feed material is restricted, the extend being determined by the OIE status (see section 3.2.5). However, in many countries the use of specific slaughter by-products, e.g. blood and fat products, is allowed. In some countries, in small scale farming, kitchen waste is one of the feed ingredients for ruminant and poultry. In small scale pig farming, food waste from restaurants and hotels is fed to pigs and pork and this feeding is considered of good quality (Osti and Mandal 2012).

Besides the production volumes, no records for the global use of FFPs were found.

Estimations of amounts of FFPs entering the feed chain are very scarce in literature. Some indications are available for the Netherlands (van Raamsdonk *et al.*, 2011). The main category of FFPs used as feed ingredient consist of bakery products, a representative of the cereal category of FFPs, which include dried and ground meal from bread and biscuit products. Biscuit meal comprises biscuits, treacle waffles, chocolate (not confectionary), gingerbread, breakfast cereals, crisps, nuts, among others. The estimated volume of recycling of bakery products in the Netherlands is approx. 300,000 MT, including imported material mainly from Germany. This is approx. 2.5% of the Dutch annually produced volume of compound feed. Dry products further include sweets and dairy powders. As far as these types of FFPs are recycled as ingredient in animal feeds, the annual volume is estimated to be approx. 40,000 MT. Sweets, originally dry products, are processed in the form of syrup by dissolving and removing packaging materials from the wet product.

The literature search did not yield any other publications on amounts of FFPs entering the feed chain in other countries.

Potential hazards

As a consequence of their original purpose, FFPs comply to a series of legal restrictions, both procedural based (HACCP guidelines; Parisi *et al.*, 2015) and product based (limits on a range of chemical and biological contaminations). Due to prolonged storage and additional processing, which includes removal of the packaging materials, primarily (micro-)biological and physical hazards can be assumed to be at stake.

For food-processing by-products of increasing relevance, the range of hazards relevant to feed could be very broad and diverse and may include potential feed contaminants like aflatoxins, heavy metals and pesticides (FAO, 2016).

FFPs can act as vector for transmitting viral diseases as well as microbial contamination. Viral and microbial diseases are a considerable hazard for farmed animals. Besides several other transmission routes for classical swine fever (CSF), such as secretions and excretions, other (wild) animals, mucus and skin contacts, transport vehicles and artificial insemination, the feeding of kitchen waste (swill) is considered a main infection route (Ribbens *et al.*, 2004; Anonymous, 2009). Nevertheless, a model with 22 parameters, excluding feed as vector, was still successfully applied to predict and explain outbreaks of CSF in Spain (Martinez-Lopez *et al.*, 2011, 2012). Bone marrow is indicated as carrier of the virus inducing foot and mouth disease (FMD). Among a range of other factors, non-deboned meat and swill can act as vector for transmitting FMD (Paton *et al.*, 2009; Hagenaars *et al.*, 2011). In order to investigate microbial safety, product types with high moisture, such as kitchen waste, dairy and fruits, were processed as feed material by applying 65 °C for 20 min. This treatment was reported to be sufficient for an appropriate microbial quality, and as side effect, for reduction of the moisture percentage (Garcia *et al.*, 2005). In another study kitchen waste was treated at 60-110 °C for up to 60 min. This appeared to be not fully sufficient for reaching sterility, since some moulds and yeasts still survive after this treatment (Jin *et al.*, 2012). Chen *et al.* (2012) applied a treatment of 120 oC for 40 min to catering waste and found complete sterilization with respect to molds and yeasts, Staphylococcus aureus, total coliforms and total aerobic counts.

Chemical hazards

A principal extra set of hazards of FFPs over other feed materials is the likely situation of having been packed or still being packed. Chemicals might probably have diffused from the packaging material (formerly indicated as food contact material, FCM) into the packed food, and unpacking might result in remnants of packaging material which might cause physical hazards.

Some groups of chemical hazards related to packaging materials include plasticizers in plastics, printing inks, softeners in cellulose, retention agents and coating, both of paper, and aluminium. An overview of backgrounds to these ingredients is given in Van Raamsdonk *et al.* (2011).

Compounds indicated as endocrine disruptors, most notably bisphenol A (BPA) and phthalates have shown to be able to induce epigenetic changes. Epigenetic modifications are known to be transmissible to next generations, and effects including ovarian disease, testis disease, pubertal abnormalities and obesity were observed in generations 1 to 3 after exposure to generation 0 rat animals. In addition, kidney and prostate abnormalities were found in generation 1 (Manikkam *et al.*, 2013). Phthalates were recognised as emerging hazards (Parisi *et al.*, 2015) and feed is one of the potential sources of introduction in the food chain. Recently, a meta-analysis of 33 studies (28 test populations) extracted from 1314 references was published (Bonde *et al.*, 2017). A limited effect was found of an overall odds ratio (OR) of 1.11 (95% confidence interval 0.91-1.35) for male reproductive disorders as chosen end points. Only for four specific compounds, all persistent organochlorine compounds, sufficient data were provided for a specific assessment. The study report mentioned that the exposure to the rapidly metabolized and excreted BPA and phthalates causes serious problems for collecting reliable data (Bonde *et al.*, 2017). This situation might limit the possibility to draw reliable conclusions from an epidemiological study.

Printing inks can contain photo-initiators and amine synergists, most notably isopropylthioxanthone (ITX) and benzophenone (BP), besides a range of other substances (Jung *et al.*, 2013). These compounds are shown to be able to migrate to the packed food, in some cases within the best before period, in the presence of a polyethylene film as protective layer, either direct or via the vapour phase (Jung *et al.*, 2013).

When considering the hazards of compounds in packaging material of FFPs to be intended as feed material, two elements are important. Remnants of packaging material might act as a direct source, while the formerly packed FFP can contain these compounds due to migration. Considering a level of 0.15% of packaging material in the unpacked FFP, the level of the compounds at stake are assumed to be too low to pose any hazard (7.5 mg/kg printing ink; van Raamsdonk *et al.*, 2011). A level of 0.15% of remnants of packaging materials was considered safe (Kamphues, 2005). With respect to the second route of exposure, the level of compounds migrated to the FFPs themselves, no literature was found.

Garcìa *et al.* (2005) reported high levels of lead, cadmium, Cd, PCBs and PCDD/F in restaurant waste and household waste, in most cases not exceeding legal limits as far as applicable. It can be assumed that edible material, initially intended for human consumption would meet the requirements for a range of undesirable compounds, including heavy metals, pesticides and contaminants. For a few specific categories, most notably mycotoxins and processing related compounds (e.g. dioxins), levels can increase during storage and processing. For example, dioxins have been found after uncontrolled processing by direct heating. Incidences are the dioxin contaminations in bakery products in Germany and in Ireland after drying without a physical separation between the product and contaminated fuel (see further section 3.1.1.1). Bakery waste may also contain semicarbazide (Noonan *et al.*, 2005) and acrylamide (Pabst *et al.*, 2005). Potato peels are used as animal feed and this use may lead to contamination with dioxins due the use of kaolinic clay in the sorting process (see section 3.1.1.1).

Adverse physical effects are related to the contamination level as well as to the particle size and shape of remnants of packaging material in FFPs: a few large particles can result in obstipation, and sharp edges can result in intestine damage (van Raamsdonk *et al.*, 2011).

A total of 243 samples of FFPs were investigated between 2005 and 2010 in the Dutch official monitoring program for feed (van Raamsdonk *et al.*, 2011, 2012). Considering an action level of 0.15% w/w of remnants of packaging material, more than 90 % of all samples investigated and over 95% of the 160 included samples of bakery products showed lower levels.

The literature search did not yield any other publications on monitoring of packaging materials in feed materials.

Transfer to food of animal origin

Transfer to animal products intended for human consumption would apply to biological and chemical contaminants. Physical hazards in food of animal origin of FFPs as feed material are absent by definition. Acrylamide from bakery waste can transfer to milk and eggs (Pabst *et al.*, 2005; Halle *et al.*, 2006). Transfer of other undesirable substances is already covered in Chapter 3 (viz. in section 3.1.1.1 for dioxins, 3.1.1.3 for organochlorine pesticides, 3.1.2 for potentially toxic elements and 3.1.3.1 for mycotoxins).

The risks of exposure to chemical compounds from remnants of packaging materials in FFPs via the animal feed route is generally low (van Raamsdonk *et al.*, 2011).

Knowledge gaps

- The presented literature shows that different views on hazards of FFPs and food-processing by-products of increasing relevance exist. Therefore, the most prominent and relevant hazards have to be inventoried, worldwide as well as regionally. A consensus or at least a harmonised view on the opportunities, limitations and hazards of the utilisation for animal feeding needs to be developed.
- With respect to viral diseases, the impact of feed as transmission vector relative to all other factors should be investigated in the framework of the restrictions of feeding certain animal proteins (see Chapter 3.2.3).

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BIOFUEL BY-PRODUCTS

Description of the feed products

The increasing demand for environmentally friendly fuels leads to increased cultivation of crops for the production of biofuels. In many cases, biofuel production yields by-products that may be used in livestock feed. This is an efficient way to deal with these by-products, but it is important to be aware of the potential hazards.

Biofuel by-products

Product	Biofuel by-products used as feed ingredients: Dried distiller's grains with solubles (DDGS) and wet distiller's grain (WDG) from bio-ethanol production (protein-rich) Crude glycerol from bio-diesel production (energy source) Plant press cakes/meals from bio-diesel production (protein-rich)
Potential hazards	**Chemical:** • DDGS/WDG: - Mycotoxins, including aflatoxins, ochratoxin, fumonisins, deoxynivalenol, nivalenol, T-2, HT-2, zearalenone and ergot alkaloids; co-occurrence of several mycotoxins is frequently found - Antibiotics, including virginiamycin, streptomycin, ampicillin, penicillin, erythromycin, tylosin, monensin and tetracycline. - Sulphate / sulphite **Crude glycerol:** • Methanol • Sodium **Plant press cakes:** • Plant toxins, e.g. phorbol esters in Jatropha curcas kernel meal, ricin in castor cake (Ricinus communis L.). • Anti-nutritional factors, e.g. glucosinolates in Brassica camelina and Brassica carinata meal, furanoflavones, tannins and trypsin inhibitors in Pongamia glabra (Karanj) cake **Biological:** WDG: growth of moulds and fungi (limited shelf life)
Potential human and animal health impact & feed-to-animal-product transfer	**Chemical:** • DDGS/WDG: - Mycotoxins: see section 3.1.3.1 - Antibiotics: (i) the concentrations are relatively low and consequently the risk that residues of the antibiotics will end up in food of animal origin are very low. Nevertheless, these low contents could potentially contribute to the development of antibiotic resistance. For further information about antibiotic resistance, see section 3.2.5 (ii) Inclusion of DDGS in medicated feed could give rise to drug interactions that could lead to potential animal hazards, but the risk is considered low due to the low concentrations of the antibiotics. (iii) Some antibiotics are very toxic for specific animals, e.g. monensin is toxic to horses and turkeys, cf. section 3.1.3. - Sulphate / sulphite: excess consumption can lead to a wide variety of adverse effects in animals. No transfer to food of animal origin. **Crude glycerol:** Methanol: at high levels toxic to ruminants. No transfer to food of animal origin. Sodium: at normal inclusion levels not expected to be a concern for animal health. No transfer to food of animal origin. **Plant press cakes:** • Plant toxins are toxic to animals, e.g. phorbol esters (PEs) in Jatropha curcas kernel meal, ricin in castor cake (Ricinus communis L.). Processes that almost completely remove or degrade PEs in Jatropha products are available. Cf. section 3.1.3.2. • Anti-nutritional factors: see section 3.1.3.2. **Biological:** • WDG: growth of moulds and fungi may lead to formation of mycotoxins. This can be prevented through short and proper storage or addition of preservatives.
Knowledge gaps	**Crude glycerol:** levels and safety aspects (including transfer to food of animal origin) of chemical contaminants and prohibited substances if input materials others than vegetable oils intended for human consumption are used for biodiesel production (EFSA, 2010). **Press cakes:** for newly introduced crops the knowledge about transfer of (residual) plant toxins to food of animal products and effects on human health is very limited. One specific example is the transfer of residues of PEs from Jatropha meal to animal derived products. More data are needed to draw firm conclusions on human risks (EFSA, 2015).

By-products from biofuel production are now available in large amounts, from different sources and in very different qualities. These by-products, including the major ones highlighted below, have been successfully included in animal diets (Pinotti and Dell'Orto, 2011; Pinotti *et al.*, 2014).

Dried distiller's grains with solubles (DDGS) and wet distiller's grain (WDG)

Historically, dried distiller's grains with solubles (DDGS) was known as a by-product of the fermentation stage in alcoholic beverage production in distilleries and traded as a high-protein feed commodity, while more recently bioethanol production has become the main source. The process of producing DDGS starts by liquefying a crop, usually maize or wheat. Yeast is added to the mash and the product is fermented to produce ethanol. The resulting mash is distilled and centrifuged to remove liquids. The solubles that have been separated from the distiller's grain in the liquid phase are concentrated by evaporation. The latter is known as "condensed distiller's solubles" (CDS) or "syrup" and can be used as a liquid feed or re-added to the centrifuged solids before drying, producing DDGS that can be used for feed.

USA, Canada and the EU-28 are the major producers of grain-based ethanol and thereby DDGS. In the USA, ethanol production is based mainly on corn, while in Canada and the EU-28, it is based on both wheat and corn. Worldwide, 6 and 124 million metric tons of wheat and coarse grains, respectively, were used in the manufacture of bioethanol in 2008–2010, which are expected to increase to 15 and 166 million tons, respectively, in the OECD-FAO forecasts for the year 2020 (OECD, 2011; Pinotti et al, 2016).

Wet distiller's grain (WDG) has a moisture content of approx. 50% (as opposed to 10-12% for DDGS) and has a much lower shelf life. WDG, produced by breweries is used mainly for dairy cattle.

Glycerol

Glycerol is a by-product of the production of biodiesel via transesterification. Pretreated and purified oil and fats enter an equilibrium reaction with a strong base to form glycerol and a group of fatty acids. These fatty acids are then esterified with methanol to produce biodiesel and water. Glycerol is separated from the crude biodiesel mash and residual methanol is removed by evaporation or distillation. Subsequently, the biodiesel mash is washed and dried to become biodiesel.

The oils and fats that are used as input materials for biodiesel production may be from vegetable or animal origin. Animal fats used for biodiesel production may include also animal by-products (ABP), including high risk (so-called Category 1) material, that potentially contains undesirable or unauthorised substances (EFSA, 2010).

Plant press cakes/meals

Multiple plants like rapeseed, palm and soya have been used in the last decades to produce biodiesel. The pressed cake that remains after cold-press oil extraction and the meal after hot solvent extraction are often used for feed owing to their high protein contents.

New crop types are being used to develop better fuel sources. New varieties recently approved in Canada include Brassica camelina sativa meal and Brassica carinata meal, both by-products from jet fuel production (Canadian Food Inspection Agency, 2014).

Jatropha curcas currently is widely grown in many tropical and sub-tropical countries for biodiesel production. The remaining cakes or meals have a high protein content (approximately 60-65% in the case of kernel meal), making them potentially valuable as an animal feed ingredient (Makkar *et al.*, 2012; EFSA, 2015). Another genotype of Jatropha, J. platyphylla, has been identified, but its distribution is restricted to a limited number of regions in Central America and this genotype is not (yet) used for oil extraction for biodiesel production or as a feed material (EFSA, 2015).

Castor oil plant (Ricinus communis L.) is produced in several countries for biodiesel production, mainly in India, China and Brazil. The use of castor seed cake as feed material is seriously restricted due to the presence of the toxin ricin (Anandan *et al.*, 2012). Other candidate plants for which research is ongoing with regard to the potential use of press cakes / meals as feed materials are Pongamia glabra (Karanj) and Azadirachta indica (Neem) (Dutta *et al.*, 2012).

Potential hazards of DDGS and WDG

A number of hazards have been reported, of which the main ones include chemical hazards, viz. mycotoxins, antibiotics and sulphate / sulphite (Lywood and Pinkney, 2012; U.S. Grains Council, 2012; Granby *et al.*, 2012; Li *et al.*, 2014).

Mycotoxins

Prevalent mycotoxins in DDGS and WDG include aflatoxins, ochratoxin, fumonisins, deoxynivalenol, nivalenol, T-2, HT-2, zearalenone and ergot alkaloids (Weaver, 2010; Granby *et al.*, 2012; Chełmińska and Kowalska, 2013; Pinotti *et al.*, 2016).

These mycotoxins can have multiple sources, including moulds that have previously infected the crop in the field or in post-harvest stages, i.e. during crop storage or throughout the process of DDGS production. This is the main reason to reduce moisture content of DDGS: WDG has a much lower shelf life due to the potential growth of moulds and yeast, for which reason preservatives can be added to produce “modified WDG” so as to prolong shelf life. It should be assured that the time between production and feed consumption of WDG is as short as possible.

The level of mycotoxin contamination in DDGS depends on the original grain contamination, processing methods, storage conditions, fermentation yeast properties, and year of production (Pinotti *et al.*, 2016). As the drying steps during bioethanol production do not include drainage, mycotoxins with relatively high water solubility are also expected to concentrate in the DDGS (Granby *et al.*, 2012).

Occurrence of mycotoxins in DDGS has recently been reviewed by Pinotti *et al.* (2016). In a survey of corn DDGS samples sourced worldwide that were analysed for aflatoxins, zearalenone (ZEA), deoxynivalenol (DON), fumonisins and ochratoxin (OTA), the main result was the high percentage of multi-mycotoxin contamination; 92% of the samples were contaminated with 2 or more mycotoxins. Of the 409 samples that were analysed, 2% exceeded the European feed limit for aflatoxin B1. The European recommended guidance values for deoxynivalenol, fumonisins and zearalenone were exceeded in 1 – 8 % of the samples (Rodrigues and Chin, 2012).

The occurrence of aflatoxins, DON, fumonisins, T-2 toxin, and ZEA contamination has been reported in DDGS samples from several corn ethanol plants in the Midwestern United States. The levels of contamination were very variable and generally lower than the advisory levels for use as animal feed provided by the U.S. FDA with few exceptions. Regarding DON, 12% of the samples contained DON levels that were higher than the advisory level. 6 % of the samples contained fumonisin levels that were higher than the maximum level recommended for feeding equids and rabbits (Zhang and Caupert, 2012).

In another survey in the U.S.A., 141 corn DDGS lots collected in 2011 from 78 ethanol plants located in 12 states were screened for several mycotoxins. Highest levels were found for DON (range from <0.50 to 14.62 mg/kg), 15-acetyldeoxynivalenol (range from <0.10 to 7.55 mg/kg) and ZEA (range from <0.10 to 2.12 mg/kg) (Khatibi *et al.*, 2014).

In samples of wheat based DDGS (n = 7) the presence of enniatin B in addition to DON and OTA has been reported. The levels of nivalenol, T-2 toxin, HT-2 toxin, 3-acetyl-deoxynivalenol, 15-acetyl-deoxynivalenol, ZEA and beauvericin were below the limit of detection. AFB1 was not included in the survey. The maximum concentrations for DON and OTA were 0.57 mg/kg and 0.007 mg/kg, respectively. The presence of enniatin B in all samples (maximum level 1.83 mg/kg) indicates that, according to the authors, the potential impact of this emerging mycotoxin on feed and food safety deserves attention (Mortensen *et al.*, 2014).

The relationship between mycotoxin concentrations in the grain and in the DDGS has recently been reviewed by Pinotti *et al.* (2016). Although a slight degradation of fumonisins during fermentation has been reported, in general mycotoxins are not destroyed during the ethanol fermentation process or during the production of DDGS. An enrichment of DON and ZEA from corn to DDGS of 3–3.5 times has been reported for ethanol industrial plants with different processing parameters. Unlike the situation for DON, the DON glucoside was not concentrated into DDGS, indicating that some DON glucoside may have been hydrolyzed during the fermentation process and that the ethanol yeasts may hydrolyse the conjugate. For fumonisin, an average increase of three times has been reported (Pinotti *et al.*, 2016).

Antibiotics

Ethanol fermentation containers can become infected with bacteria that compete with yeast for sugars and micronutrients competitively inhibiting bioethanol production during the fermentation process. Antibiotics are often used in the process to decrease the level of organic acid producing bacteria. This could result in residues of antibiotics in distiller's grain by-products.

A number of antibiotics are used in the production of bioethanol, including virginiamycin, streptomycin, ampicillin, penicillin, erythromycin, tylosin, monensin and tetracycline. Of these, in the U.S.A. virginiamycin and penicillin are the most commonly used. When antibiotics are used, they are added to fermenters in small concentrations. For example, virginiamycin is typically added at levels of 0.25 to 2.0 µg/g (Shurson *et al.* (2012; U.S. Grains Council, 2012). In Canada, an approved list of antibiotics has been elaborated, based on health risk assessments. Depending on the active substance, the maximum inclusion rates are up to 6 µg/g (Canadian Food Inspection Agency, 2014). The use of monensin and tylosin is not allowed in Canada, due to monensin's known toxicity to horses and turkeys, and the high potential use rate of DDGS in tilapia feed leading to excessive exposure to tylosin (Canadian Food Inspection Agency, 2017).

In a survey conducted in the U.S.A. in 2011 on 80 DDGS and 79 WDG samples 13 % of the samples contained antibiotic concentrations with a maximum of 1.12 µg/g. Erythromycin was found in 16 of the samples (10.1%), concentrations were less than 0.8 µg/g. Two samples were positive for virginiamycin (0.6 µg/g and 0.5 µg/g). One sample contained tetracycline and one sample contained penicillin. None of the samples contained tylosin residues (Paulus Compart *et al.*, 2015; U.S. Grains Council, 2012). In 2012, the FDA conducted a survey to check for 13 antibiotic residues. Of the total of 46 samples analysed, 3 samples contained detectable levels of erythromycin, virginiamycin, and penicillin with concentrations ranging from 0.15 to 0.58 µg/g (U.S. Grains Council, 2012).

Since the concentrations that are found in DDGS and WDG are relatively low compared to the contents that are allowed in various parts of the world for inclusion in animal feed, the chance that residues of the antibiotics will end up in food of animal origin are very low. With reference to an in-vitro study by Ge *et al.* (2017), the possible implications of such low antibiotic residue levels for the occurrence of antibiotic resistance are also discussed in section 3.2.5.

Furthermore, drug interactions could lead to potential hazards, e.g. for the combination of monensin in medicated feed and DDGS containing erythromycin residues, where cattle became ill or died. It should be noted that the concentrations of erythromycin were several orders of magnitude greater (50 to 1500 µg/g) than erythromycin concentrations found in the abovementioned surveys (Paulus Compart *et al.*, 2015).

Sulphate / sulphite

During production of bioethanol, sulphuric acid is added during fermentation to keep the pH low and to keep the distillation columns clear of precipitate. The concentration of sulphurous compounds in DDGS, mostly as sulphate ions and a to a small extent as sulphite ions, was reported to range from 3.9 to 11.4 g/kg dry matter (Li *et al.*, 2014). Excess consumption of these sulphurous compounds (above 4 g/kg diet DM) from feed and water can lead to polioencephalomalacia (PEM) and sulphur toxicosis. These illnesses can contribute to a wide variety of adverse effects, like blindness or inflammatory digestive disorders (Ensley, 2011; Neville *et al.*, 2012; Amat *et al.*, 2014).

Potential hazards of biodiesel by-products

Contaminants of glycerol

There are two types of glycerol available: purified glycerol is used in food and crude glycerol (containing ± 15% impurities) is used in feed. Regular practice is the addition of 10% of crude glycerol as dry matter in feed (EFSA, 2010; Hippenstiel *et al.*, 2012).

The main impurities in crude glycerol are methanol and sodium. Other impurities may include ethanol, sulphate and phosphorous compounds. Methanol removal from glycerol may be incomplete. In the United States, the maximum limit for methanol in crude glycerol for safe use in feed is 5000 µg/g (Association of American Feed Control Officials, 2016). In the European Union, also a maximum level of 5000 µg/g is included in the specifications laid down in the EU Catalogue of feed materials (European Union, 2017). This level of contamination does not represent a risk for animal health at a total inclusion level of 10 % in the diet of monogastric animals and 15 % in the diet of ruminants (EFSA, 2010). The actual methanol level varies considerably per sample and is in some cases toxic to ruminants (Coma, 2010; Ensley, 2011).

Sodium (originating from sodium hydroxide in the alkalinisation process) is found in the crude glycerol fraction up to a level of 1 %. At normal inclusion levels, these amounts contribute only marginally to the daily sodium intake of the animals. This increase is not expected to be a concern for animal health (EFSA, 2010).

No transfer of methanol or sodium has been identified to foods of animal origin. Consequently, there is no impact on human health (EFSA, 2010).

If input materials for biodiesel production other than vegetable oils intended for human consumption are used (e.g. Category 1 ABP or unconventional oils containing toxins), these materials may contain potentially hazardous substances that may co-elute into the crude glycerine fraction, such as environmental contaminants (e.g. heavy metals) mycotoxins, plant toxins, residues of veterinary medicinal products, substances having a hormonal or thyrostatic action and beta-agonists. Thus, the use of these materials remains of concern to human and animal health unless it is proven that the chemical processes involved in the trans-esterification of the feedstock in the biodiesel production inactivate these chemical contaminants (EFSA, 2010).

Toxins in plant press-cakes

In Canada, Brassica camelina meal and Brassica carinata meal have been assessed with respect to animal and human health. Challenges with the assessment process included the evaluation of higher levels of antinutritional factors, viz. glucosinolates, associated with these plant types. Maximum inclusion rates and restrictions regarding animal species were established to protect animal safety (Canadian Food Inspection Agency, 2014).

Untreated Jatropha curcas kernel meal contains toxic phorbol esters (PEs) in concentrations varying between 600 and 3,700 mg/kg and also anti-nutritional substances, making it unsuitable for use as a feed ingredient. Processes that almost completely remove or degrade toxic PEs in Jatropha products are available, resulting in levels below the limit of detection of 3 mg PEs/kg (Makkar *et al.*, 2012; EFSA, 2015). EFSA concluded that after detoxification, Jatropha material would not pose a health risk to pigs while the risk to other species is likely to be low. The transfer of Jatropha PEs to animal derived products is unknown. More data are needed to draw firm conclusions on human risks (EFSA, 2015). J. platyphylla is free of toxic phorbol esters; however, its seed kernels and kernel meal contain trypsin inhibitors, lectin and phytate (Makkar *et al.*, 2012).

Some of the plants cultivated for biodiesel production contain highly toxic components. Ricin, for example, is a ribosome-inactivating protein from the seed beans of the castor oil plant (Ricinus communis L.). It is composed of two polypeptide chains, one with toxic activity and the other with cell-surface-binding activity, interconnected by a disulphide bond. After uptake of ricin by cells, reduction of the disulphide bond leads to release of the toxic chain from its counterpart, and subsequently to its toxic, ribosome-inactivating action within the host cell. Ricin is very toxic to both humans and animals. Research conducted so far has shown that processed castor cake can certainly be incorporated at low levels in ruminant feeds, and with better processing methods higher levels of incorporation are possible. However, until now the technologies for detoxification of castor seed cake have not been adopted by industry or otherwise commercialized (Anandan *et al.*, 2012).

A limited number of other plants contain similar ribosome-inactivating proteins, the most important being Abrus precatorius L. and Croton tiglium L., which contain abrin and crotin I, respectively. When these plants are used in the production of biodiesel, the toxins are concentrated in the press-cakes. These press-cakes are in some countries used in animal feed. Ricin, abrin and crotin are highly toxic and are deadly when ingested in small doses; small amounts of raw castor beans, for example, are sufficient to cause serious harm to livestock. Due to their high toxicity, chances of human ingestions through animals is minimal and, when properly cooked, these proteins will degrade rapidly (EFSA, 2008; Anandan *et al.*, 2012).

Karanj (Pongamia glabra) cake toxins include furanoflavones (karanj, pongamol, pongapin, pongaglabron, kanjone, isopongaflavone, lanceolatin B), tannins and trypsin inhibitors. Neem (Azadirachta indica) seed cake contains toxic triterpenoids (azadirachtin, salanin, nimbin, nimbidiol) and its bitterness is attributed to these compounds. De-oiling of karanj cake results in complete removal of fat-soluble toxic compounds, and water washing of karanj cake and neem seed cake can detoxify them partially (Dutta *et al.*, 2012). According to other authors, karanj seed cake, even after defatting and detoxification, has to be used with great caution due to adverse effects on growth rate and testicular architecture caused by residual toxins like karanjin (Dinesh Kumar *et al.* 2013; Rao and Dinesh Kumar, 2015).

For more information on plant toxins, see section 3.1.3.2.

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AQUATIC PRODUCTS OF ANIMAL ORIGIN

Description of the feed products

With the consumers' demand for fish increasing, the amount of worldwide aquaculture has increased steadily over the last twenty years. At the same time, the amount of fish caught for production of aquaculture feeds has levelled off due to fishing quotas and environmental issues (Roest, Vos and Marvin, 2010). Besides the rising use of crop-derived oils and protein-rich ingredients in aquaculture feed formulation, novel feed ingredients include the use of discarded species, bycatch and fish waste from processing plants, and the use of macroplankton, specifically krill products, given their high abundance (Gillund and Myhr, 2010). Other potentially interesting applications include the use of fish silages as aquatic and terrestrial animal

Aquatic products of animal origin

Product	Aquatic products of animal origin used as feed ingredients, e.g. rich in protein, oil Hydrolysates and silage from fish by-products, waste and other fish Krill oil and meal
Potential hazards	**Chemical:** • Fish hydrolysates & silages: - Contaminants that bio-accumulate through aquatic food webs (such as persistent organic pollutants including dioxins, PCBs, flame retardants, chlorinated pesticides) and others that tend to accumulate in fish (e.g. potentially toxic elements); - Preservatives and their impurities and metabolites, such as BHT and ethoxyquin that are used to prolong storability of fish hydrolysates and other fish products used for feed production, and which can be transferred from feed to food products of animal origin • Krill and derived products: - High fluorine levels (livestock animal health hazard) **Biological:** • Fish hydrolysates & silages: Zoonotic pathogens present in input materials such as dead fish from earth ponds, particularly those that can survive processing conditions, e.g. Clostridium botulinum (spore-forming) **Physical and other:** • Fish hydrolysates & silages: Nano and microplastics: may be transferred to tissues of marine organisms used as feed ingredients
Potential human and animal health impact & feed-to-animal-product transfer	**Chemical & biological:** • See chapter 3 for hazards already covered, such as persistent organic pollutants (including dioxins, non-dioxin-like PCBs, brominated flame retardants, organochlorine pesticide residues) and potentially toxic elements (section 3.1.1), and spore-forming microorganisms (e.g. Clostridium, see section 3.2.1) • Fluorine in krill: - Excess fluorine is known to have a high impact on livestock animal health such as stunted growth, skeletal deformations. - Transfer to food products of animal origin is low and does not contribute significantly to consumer exposure **Physical:** inconclusive and only limited data, e.g. on uptake and toxicity of mjcro- and nanoplastics in target livestock species
Knowledge gaps	• Physical and chemical: uptake and toxicity of plastic nano- and microparticles from aquatic products of animal origin (e.g., filter-feeding shellfish) ingested as feed ingredients by livestock species • Biological and chemical hazards of relatively new ingredients such as krill, fish species and fish parts not traditionally processed into feed • Occurrence and behavior of certain acid-resistant biological and chemical hazards (viruses, spore-forming bacteria, thiaminase) in fish hydrolysates and silage particularly if no heating is applied during production

feeds. Such silage methods can be applied also to fish or fish parts not commonly used for food and feed purposes, such as viscera from fish processing or invasive fish species (Haider, Ashraf, Azmat *et al.*, 2015; Tejeda-Arroyo, Cipriano-Salazar, Camacho-Díaz *et al.*, 2015). During the process, the fish or fish parts are acidified either through fermentation or through addition of mineral of organic acids, and thereby rendered storable for prolonged periods. Storability may also be further enhanced by subsequent addition of preservatives such as BHT and ethoxyquin and other antioxidants, whilst these and their impurities (e.g. the mutagenic p-phenetidine in ethoxyquin) and metabolites, in turn, can be transferred from feed to food products of animal origin (EFSA, 2015; Nieva-Echevarría, Manzanos, Goicoechea *et al.*, 2015). In addition to emerging hazards in feed, alterations in the composition of feed can also change the nutritional value, but this will not be discussed further in this section.

Potential hazards in hydrolysates and silage from fish by-products, waste and other fish, used in feed

Biological hazards

Often fish that is designated as bycatch caught on fishing trips is defined as non-relevant species or juvenile specimen that are not suitable for sale. Usually these fish are thrown back into the ocean or sea, though a small quantity of this bycatch is accidently transported back to land. In order to find a profitable use for this bycatch, it is hydrolysed, grained and stored as a source of aquaculture feed (Gillund and Myhr, 2010; Khosravi, Herault, Fournier *et al.*, 2014). The same protocol is applied for fish parts found in waste water of fish processing plants. Due to the intensive protocol to produce fish hydrolysate, microbiological hazards in fish hydrolysates are less relevant (Guérard, Decourcelle, Sabourin *et al.*, 2011; Thorkelsson, Slizyte, Gildberg *et al.*, 2009). As regards the feed use of acidified fish silage from e.g. fish viscera discarded during fish processing, heat treatments (≥85°C) following the acidification step have been proposed in order to control most of the microbiological hazards. Survival is still possible, though, for spores from, e.g. Clostridium botulinum if present in the starting materials such as dead fish in aquaculture operations in earth ponds, albeit with low probability if such dead fish are removed on a daily basis [e.g. (VKM, 2010)]. Given that the heating step may help eradicate certain hazards that may survive the acid ensiling conditions, such as spore-forming bacteria, acid-resistant viruses, and thiaminase (an enzyme present in some fish species, degrading thiamine, i.e. vitamin B1).

Chemical hazards

Chemical hazards include the accumulation of potentially toxic elements like mercury, arsenic, selenium, lead and cadmium in fish (Amlund, Lundebye and Berntssen, 2007; Sapkota, Sapkota, Kucharski *et al.*, 2008) and the accumulation of dioxins, dioxin-like PCBs and NDL-PCBs in fish adipose tissue (Thorkelsson *et al.*, 2009). More information on these topics can be found in sections 3.1.1.1 (Dioxins and dioxin-like PCBs), 3.1.1.2 (NDL- PCBs), and 3.1.2 (Potentially toxic elements).

Potential hazards of nano- and microplastics taken up by aquatic animals used for feed production

Physical and chemical hazards

High concentrations of plastic debris have been observed in the oceans. This is caused by commercial shipping, fishing and other activities in the oceans, but also due to increased release of micro- and nanoplastics through sewage or waste discharge that is caused by the increased occurrence of plastic particles in cosmetics, packaging and cleaning products over the last decades. Much of the recent concern has focussed on microplastics. Because trophic transfer of microplastics undoubtedly takes place, clearly nano- and microplastics can end up in fish and thus in fish-derived products such as fish meals and hydrolysates. Microplastics are, because of their size (> 1 µm), not likely to be transported across cellular membranes, but as they might be present in the gut content, microplastic might end up in products of fish processing. As for nanoplastics, in a recent review (Bouwmeester, Hollman and Peters, 2015), it is concluded that the currently used analytical techniques introduce a great bias in the knowledge since they are only able to detect plastic particles well above nano-range. Not much is known about the possible adverse effects nanoplastics could have on humans. The assessment of hazards caused by micro- and nanoplastics in fish is complicated because the doses, surface shapes, material toxicity and persistence of nanoplastics may all be factors in determining hazardous biological effects (Klinger and Naylor, 2012; Liu, Tourbin, Lachaize *et al.*, 2014; Mattsson, Ekvall, Hansson *et al.*, 2015). Two possible toxic effects are recognized: the potential toxicity of the nanoplastic particles themselves, and the release of adhering persistent organic pollutants (POPs) and leachable additives from these particles. Local effects on the gut epithelium (of environmental species but also humans) and the liver should be studied. The effects of nanoplastics on the gut epithelium might affect the barrier capacity of the gut wall also for other chemicals. In section 4.7, more information on nanoparticles can be found.

Potential hazards of krill in feed

Chemical hazards

To accommodate the need for alternate sources of animal feed, and especially marine feed, Antarctic and North Atlantic krill is also being investigated as a potential feed source due to its high availability and high content of omega-3 fatty acids (Gillund and Myhr, 2010; Klinger and Naylor, 2012). Chemical hazards associated with krill are largely similar to that of other marine organisms used in feed. In addition to these possible hazards, the shell of krill contains relatively large amounts of fluoride compared to conventional fish feed. It has been shown that fluoride can accumulate in the vertebral bone of different fish, inhibiting the growth. The exoskeleton has to be removed first before krill can be processed into feed, krill products containing low amounts of fluoride can be beneficial to fish without having effects on growth (Ramprasath, Eyal, Zchut *et al.*, 2014). Inadvertent, chronic intake by cattle and other livestock of excess fluoride, for example from contaminated drinking water, pastures and forage crops with deposits of soil or volcanic ash high in fluorine, forage crops grown on fluorine-rich soil, and compound feeds that have not been defluorinated is known to adversely affect animal health, particularly in ruminants and horses, and less in poultry (EFSA, 2004; IPCS, 1984). Health impacts on livestock include, for example, reduced milk yield and skeletal deformations, as well as lameness (EFSA, 2004; IPCS, 1984). As regards the transfer of fluorine from krill-based feed to edible fish parts, Moren *et al.* (Moren, Malde, Olsen *et al.*, 2007) observed that various fish species fed on experimental diets containing elevated fluorine levels following the inclusion of krill or amphipod meal did not show accumulation of fluorine in their organs. The EFSA Scientific Panel on Contaminants in the Food Chain concludes that, in general, the fluorine absorbed by livestock animals accumulates in calcified tissues and that transfer to edible products of animal origin is low and does not contribute significantly to human exposure (EFSA, 2004).

Knowledge gaps

The research on krill used for feed is relatively new, which means that potential hazards may be discovered in the future. In this search, no information on potential microbial and physical hazards in combination with krill in fish feed was found.

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AQUATIC PRODUCTS OF PLANT ORIGIN

Description of the feed products

A large group of non-flowering plants growing in fresh water and marine environments are indicated by the gross term "algae". Exploration of marine biomass, primarily macro-algae (van den Burg, Stuiver, Veenstra *et al.*, 2013) and of fresh water biomass from micro-algae, primarily for energy production or selected products (Enzing, Ploeg, Barbosa *et al.*, 2014), is increasing strongly. Both macro- and micro-algae have already found applications in animal nutrition. For example, meal from marine macro-algae

Aquatic products of plant origin

Product	**Algal products** • Macro-algae (seaweed) used as feed ingredients or additive (e.g. as iodine-rich supplement) • Microalgae or their protein-rich biomass retained after oil extraction used as feed commodity (e.g. Spirulina) • Omega-3-PUFA-rich oil from microalgae as fish feed ingredient
Potential hazards	**Chemical:** • Inorganic: - Iodine, which can be present at high levels in macro-algae (seaweed) - Arsenic, of which both the organic and inorganic (particularly toxic) forms concentrate in seaweeds (e.g. Hijiki) and microalgae. - Other heavy metals, particularly cadmium, since these are taken up by algae from water • Organic: - Environmental residues of pesticides and other persistent organic pollutants, such as dioxins, lectins, phlorotannins and other phenolics, naturally produced by seaweeds - Organic forms of heavy metals, e.g. methyl-mercury - Toxins from toxin-producing microalgae (e.g. harmful algal blooms) adventitiously present in aqueous environment, e.g. co-harvested with macro-algae **Biological:** • Bacterial pathogens such as fecal zoonotic pathogens. Such pathogens could originate, for example, from run-off and discharge feeding into estuarine waters, which can then be taken up by algae acting as reservoir. This would particularly also apply to the case of microalgae being used for wastewater treatment **Physical:** • Micro- and nanoparticles taken up from aqueous (e.g. marine) environment
Potential human and animal health impact & feed-to-animal product transfer	**Chemical:** • See chapter 3 for hazards already covered, such as heavy metals, organic pollutants and nanoparticles • Iodine in seaweed: - May cause hyperthyroidism in animals and humans - Transfer to edible parts and products (e.g. milk and eggs) of the animal will occur to a substantial extent • Phlorotannins: Reduced ruminal protein degradability in ruminants **Biological:** Pathogens transferred from nutrient sources (e.g. manure, wastewater) used in micro-algal conversion to products or by-products used as animal feed **Physical:** Inconclusive with few data on uptake and on toxicity in target livestock species
Knowledge gaps	• Lectins and other intrinsic toxicants and anti-nutrients produced by seaweed species: occurrence, effects, and processing stability (animal health issue) • Occurrence in algae and toxicity of plastic nano- and micro-particles • Residues of persistent organic pollutants in macro-algae not grown under controlled conditions: occurrence and levels • Occurrence of natural toxins of e.g. cyanobacteria (microcystins) in candidate species of algae for food and feed production • Accumulation by seaweeds (marine macro-algae) of phycotoxins from harmful algae adventitiously present in the vicinity of seaweed harvested for food and feed purposes • Biological contamination of seaweed meal used as e.g. aquaculture feed with bacterial zoonotic pathogens (e.g. Vibrio)

(seaweed) is used as feed ingredient in shrimp aquaculture (Cárdenas, Gálvez, Brito *et al.*, 2015) and is also more generally recognised as feed ingredient for farmed animals. Current utilisation of various species of micro-algae, mainly freshwater algae, is intended to produce certain food and feed additives and ingredients, such as ω-3 polyunsaturated fatty acids and carotenoid pigments, as well as dietary supplements. Further applications in feed are in development (Enzing *et al.*, 2014). It is necessary to distinguish three main species groups for a proper discussion of their use and hazards, i.e. brown, red and green algae. In addition, blue-green algae are also indicated as "algae", but have a different biologic position. They produce a wide range of natural toxins and currently have limited application (Stewart, Seawright and Shaw, 2008). Species belonging to other groups are being utilised as well, e.g. Schizochytrium, related to the brown algae, used for the production of oil (Jiang, Fan, Tsz-Yeung Wong *et al.*, 2004). This section will focus on four main groups of algae as summarized in Table 8. The marine macro-algae of three of these groups together are also indicated as "seaweed".

Potential hazards

The main hazards with respect to consumption of algae include heavy metals, iodine, pesticides, marine toxins, lectins and unintentional co-harvesting of harmful micro-algae, and micro- and nanoparticles. With respect to some heavy metals, it is necessary to discriminate between organic and inorganic forms. The inorganic forms of arsenic and the methyl-mercury form are the most toxic ones for arsenic and mercury, respectively (see also section 3.1.2 on potentially toxic elements).

Macro-algae

Chemical hazards

The mineral composition varies according to the algal species group (Holdt and Kraan, 2011), and additionally to seasonal and environmental circumstances [e.g. (Cavas, Cengiz and Karabay, 2012)]. A range of risk assessments have been published by the European Food Safety Authority. With respect to contaminants that might occur in marine macro-algae (seaweed), the relevant assessments apply to iodine, arsenic, cadmium, mercury and pesticides [(EFSA, 2009a; EFSA, 2009b; EFSA, 2012a; EFSA, 2012b; EFSA,

Table 8: Overview of major groups of algae with some characteristics, examples and applications.

Group	Habitat	Type	Examples	Purpose
Blue-green algae (Cyanobacteriae)	Marine, freshwater, terrestrial	Micro-algae	Spirulina	Dietary supplement, colour additive
Brown algae	Marine	Macro-algae	Kelp, hijiki	Dietary supplement, fucans, laminarin
Red algae	Marine	Macro-algae	Dulse, laver	Dietary supplement, Carrageenan, agar
Green algae	Marine, freshwater	Macro- and micro-algae	Sea lettuce, grass kelp	Dietary supplement, energy source

Note: a novel application of algae, either macro- or micro-algae, is carbon sequestering. Biomass resulting from these attempts can be used as biofuel or can enter the food supply chain (Chung, Oak, Lee *et al.*, 2013).

2012c), see also section 3.1.2 on potentially toxic elements]. A very elaborate risk assessment was published for arsenic (EFSA, 2009a). Sea products used for feed and food appeared to be the major source of human exposure to arsenic. Levels used as basis for the risk assessment for total arsenic in algae as food are: median = 24 mg/kg, average = 30.9 mg/kg, P95 [1]= 102.2 mg/kg, maximum = 236 mg/kg (n=448) [(EFSA, 2009a); see also Rose, Lewis, Langford *et al.* (2007)]. Sargassum fusiforme (Hijiki) has been reported to exceed the specific EU limit for seaweed of 2 mg/kg for inorganic arsenic with levels up to 94 mg/kg (Holdt and Kraan, 2011). Metallothionein is a sulphur-rich protein in seaweed with a high binding capacity for arsenic (Ngu, Lee, Rushton *et al.*, 2009). In general, the bioaccumulation capacities of seaweed can result in high levels of primarily heavy metals in selected products. Arsenic is primarily accumulated as arsenosugars in marine algae (EFSA, 2009a). Plants of the genera Sargassum, Padina, Dictyota (brown algae), Enteromorpha and Ulva (green algae) can successfully absorb high levels of iron, zinc and manganese (Chakraborty, Bhattacharya, Singh *et al.*, 2014, Jackson, 2006). Other studies report on bioaccumulation by seaweeds of chromium, cadmium and lead (Hou, Liu, Zhao *et al.*, 2012; Tamilselvan, Saurav and Kannabiran, 2012). Seaweeds are also known to accumulate iodine to high levels (Makkar, Tran, Heuzé *et al.*, 2016), for which reason they may be used as feed mineral supplements [e.g. (Rey-Crespo, López-Alonso and Miranda, 2014)].

Pesticide residues are occasionally found in seaweeds (EFSA, 2012b, Lorenzo, Pais, Racamonde *et al.*, 2012). Sequestered minerals produced from algae were shown to contain high levels of dioxins but this seems to be an isolated case (Ferrario, Byrne, Winters *et al.*, 2003; Hoogenboom, Traag, Fernandes *et al.*, 2015). Hydroxylated and methoxylated polybrominated diphenyl ethers (OH-PBDEs and MeO-PBDEs) and polybrominated dibenzo-p-dioxins (PBDDs) were found in red algae living in the Baltic Sea. These PBDDs and OH-PBDEs and MeO-PBDEs are most likely of natural origin (Malmvärn, Zebühr, Kautsky *et al.*, 2008).

Lectins have been reported to be present in seaweeds (Holdt and Kraan, 2011). Certain lectins are known to belong to the most toxic components in nature (Van Damme, Lannoo and Peumans, 2008). Other intrinsic compounds that may interfere with nutrition include phlorotannins, These polymers of phloroglucinol, a phenolic compound, occurring in brown macro-algae and other seaweeds. High levels of these compounds interfere with protein digestion in ruminants [e.g. (Belanche, Jones, Parveen *et al.*, 2016)]. This interference with digestion by phlorotannins is similar to that by tannins from terrestrial plants, which form complexes with feed proteins.

Marine toxins are produced by dinoflagellates and blue-green algae and can cause severe health problems. They are known to be accumulated by filter-feeders such as shellfish and clams (Gerssen, Pol-Hofstad, Poelman *et al.*, 2010). Another source of this hazard can be marine-toxin-producing micro-algae growing in mixed cultures with macro-algae. The paralytic shellfish toxin producing blue-green algae Lyngbya, for example, was found to be able to grow in mixed cultures with some green algae (Ulva and Cladophora) (Foss, Phlips, Yilmaz *et al.*, 2012). There is therefore a possibility that these toxic micro-algae are unintentionally harvested together with the macro-algae. In addition, it has been postulated that macro-algae can also accumulate the marine toxins produced by micro-algae. The freshwater macro-algae Cladophora fracta, for example, was shown to take up a cyanobacterial microcystin (Mitrovic, Allis, Furey *et al.*, 2005).

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[1] P95: the 95-percentile is the value for which 95% of the analysed samples show a lower level. Percentiles can be calculated for other percentages.

Biological hazards

Bacterial pathogens may be taken up by seaweed from the marine environment. In a recirculating shrimp cultivation system, the levels of various pathogenic Vibrio species correlated with the density of red seaweed fed into the system (Brito, Chagas, da Silva *et al.*, 2016), which warrants further research.

Physical hazards

Recent information shows relatively high levels of micro- or nanoparticles of plastic in seas and oceans. Harvesting wild or cultivated seaweeds could include certain levels of plastic particles (van den Burg *et al.*, 2013). More information about possible adverse effects of micro- or nanoparticles can be found in Section 4.4, Aquatic products of animal origin.

Micro-algae

Chemical hazards

As is known from harmful cyanobacterial blooms, various species of cyanobacteriae (blue-green algae) are known to produce toxins called cyanotoxins. The cyanotoxin group includes neurotoxins, hepatotoxins, cytotoxins and others. They comprise different chemical classes, including cyclic peptides (e.g. microcystins, such as the hepatotoxic MCYST-LR), alkaloids (e.g. saxitoxin, a paralytic shellfish poison), and the modified amino acid β-methylamino-L-alanine (BMAA) [e.g. (Enzing *et al.*, 2014)]. With regard to cyanobacteria used for food and feed purposes, recent data indicate the occurrence of cyanotoxins in commercial products derived from cyanobacteria [e.g., (Roy-Lachapelle, Solliec, Bouchard *et al.*, 2017)].

Hydroxylated and methoxylated polybrominated diphenyl ethers (OH-PBDEs and MeO-PBDEs) and polybrominated dibenzo-p-dioxins (PBDDs) were found in cyanobacteria living in the Baltic Sea. These compounds are most likely of natural origin (Malmvärn *et al.*, 2008).

Biological hazards

Microalgae can be used to recuperate valuable compounds from waste products, some of which may also be sources of faecal pathogens if not properly handled. Examples include manure from livestock operations used for recuperation of phosphate, and the digestate of biogas production used for production of animal feed [e.g. (Monlau, Sambusiti, Ficara *et al.*, 2015; Zhou, Hu, Li *et al.*, 2012)].

Transfer to food of animal origin

Transfer of undesirable substances is already covered in Chapter 3 (viz. in section 3.1.1.1 for dioxins, 3.1.1.3 for organochlorine pesticides and 3.1.2 for potentially toxic elements). Iodine occurs at high levels in in marine macro-algae, from which substantial transfer to products such as milk and eggs can occur if used as feed (Rey-Crespo *et al.*, 2014). Iodine is an essential nutrient, yet high intake levels are linked with hyperthyroidism (goiter) (EFSA, 2005). It is primarily through consumption of milk and eggs that consumers are exposed to iodine in foods from animal origin originating from feed. For high-consuming subpopulations of adults and toddlers, the maximum allowed iodine concentrations in complete feeds for dairy cattle and laying hens could pose health risks according to very conservative scenarios (EFSA, 2013).

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FURTHER READINGS

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GENETIC MODIFICATION AND SYNTHETIC BIOLOGY

Description of the feed products

Since the first large-scale commercial introduction of genetically modified (GM) crops in the mid-90s of the previous century, the global acreage planted to these crops has been rising almost continuously, with 185 million hectares grown worldwide in 2016 (James, 2016). Most of these crops have been modified with agronomically important traits such as herbicide tolerance and insect resistance particularly in the major commodity crops, including soybean, maize, cotton and oilseed rape. Besides these crops, the range of other GM staple crops, fruits and vegetables currently on the market is likely to expand in the near future, as are other traits such as nutrient enrichment, fungus-and virus resistance, and drought stress resistance (James, 2016). Whilst some of these crops may be grown for feed purposes only (e.g. maize forage), the co-products of other varieties of these crops processed for food purposes, such as gluten or press cake retained after oil or starch extraction, are commonly used as protein-rich livestock feed ingredients.

Besides the GM crops already commercialized or nearing commercialization, the field of genetic modification has recently witnessed an important development in that a growing number of new breeding techniques is becoming available to the plant breeder. Among these techniques, particularly interesting are new gene-editing methods employing DNA-cutting enzymes linked to protein or polynucleotide domains recognizing target DNA sequences. Well-known examples are transcription activator-like effector nucleases (TALENs), zinc finger nucleases (ZFNs), and clustered regularly interspaced palindromic repeats (CRISPR) – CRISPR-associated protein 9 (Cas9) (Malzahn, Lowder and Qi, 2017). Particularly for CRISPR Cas9, the technical inputs and time required to achieve results are substantially less

Genetic modification and synthetic biology

Product	• Genetically modified (GM) crops used directly (forage, seed) or indirectly (processing co-product) as feed ingredients • Substances (e.g. amino acids) and enzymes (e.g. fiber-dissolving enzymes, phytase) produced by and purified from cultures of GM micro-organisms within industrial facilities, and added to feed for nutritional purposes • Future products such as synthetic proteins obtained through advanced methods of design and genetic engineering (synthetic biology)
Potential hazards	• No hazards for currently commercialized products as they are regulated products undergoing pre-market safety assessments • GM crops developed for local markets in a particular country for which no regulatory safety assessment has been carried out in export markets for commodities in which the GM crop may occur adventitiously at low levels caused by unintended admixture
Potential human and animal health impact & feed-to-animal product transfer	• No adverse impacts known for GM feed products as they are subject to pre-market safety assessment
Knowledge gaps	• Regulatory status and requirement for pre-market safety assessment of future products of modern gene-editing and other advanced biotechnological design & engineering methods (synthetic biology) for which no conventional non-GM counterpart with a history of safe use exists. • Status and risk of commingling with internationally traded feed commodities, of locally developed GM products for which no global regulatory approval is pursued

than for traditional methods of genetic modification, whilst offering a high precision and without any linkage drag, i.e. the introduction of undesired traits from non-elite varieties as a result of conventional breeding (Kok, Keijer, Kleter *et al.*, 2008; Lusser, Parisi, Plan *et al.*, 2012). With these techniques, precise, small modifications in the target DNA can be made, which will ultimately lead to newly expressed desired traits. They also allow, for example, for the introduction of multiple mutations in multiple alleles at once, potentially affording more extensive and complex alterations of plant varieties. In addition, TALEN and CRISPR Cas9 can also be used for the introduction of insertion of foreign DNA and deletions of larger fragments of intrinsic DNA.

Whilst the commercialized GM crops so far have been modified with foreign genes introduced into their nuclear genomes, various other routes for genetic modification of plants have been exploited, such as for the creation of "plant factories". Plant cell chloroplasts can serve, for example, as an alternative target to nuclear DNA for genetic modification, allowing for high protein expression levels given the high number of these organelles per cell, lack of epigenetic impacts, and amenability towards combination of multiple single gene traits (Daniell, Kumar and Dufourmantel, 2005, Wang, Yin and Hu, 2009; Zhu, Li, Vossen *et al.*, 2012). In addition, recent improvements in the efficiency of transient expression of e.g. recombinant plant viruses or T-DNA plasmids infiltrated into harvested tobacco leaves enable the production of high quantities of proteins of interest whilst avoiding the lengthy procedure of creating a stable parental GM line for breeding (Jin, Wang, Zhu *et al.*, 2015; Mardanova, Blokhina, Tsybalova *et al.*, 2017). Other examples relevant to animal feed applications are modifications for increased biomass or biofuel production in crops and algae, of which co-products could conceivably be processed into feed (Wani, Sah, Sági *et al.*, 2015). It should be noted that these techniques are still in the experimental stage and that the same considerations as for other GM organisms may still apply.

Besides plants, GM micro-organisms, such as fungi (e.g. *Trichoderma reesei*, *Aspergillus niger*), yeast (e.g. *Saccharomyces cerevisae*), and bacteria (e.g. *Bacillus licheniformis*, *Corynebacterium glutamicum*, *Escherichia coli*), have an important, indirect role in animal feed production, namely as producers of feed additives. Main categories of such feed additives include amino acids (Leuchtenberger, Huthmacher and Drauz, 2005) and feed enzymes including fibrolytic enzymes such as xylanases, β-glucanase, amylases, and cellulases; phytate-degrading phytase (Gifre, Arís, Bach *et al.*, 2017). Usually, these products are produced during fermentation processes within contained industrial facilities, whilst the product of interest is purified from these cultures without remnants of viable micro-organisms before being processed into a commercial feed product. For the future, it is envisaged that also other products of GM micro-organisms, for instance synthetic proteins and fatty acids, will become available as feed materials (Gillund and Myhr, 2010). Interestingly, these authors indicate that experimental feeds have been composed with up to 20% bacterial proteins (Gillund and Myhr, 2010). The current status of this development is not clear from the scientific literature, for the time being the costs may be prohibitive.

Besides GM plants, there have also been recent developments in the area of GM livestock animals, both terrestrial and aquatic species, including pigs, cattle, goat, chicken and salmon & other fish. Popular targets for modification of experimental animals include increase productivity (e.g. growth enhancement), quality of food products of animal origin (e.g, meat and milk composition), disease and stress resistance (Forabosco, Lohmus, Rydhmer *et al.*, 2013). For growth enhancement, for example, various experimental animal species (pig, cattle, fish) have been genetically modified with genes encoding exogenous growth hormone, which are expressed in other tissues than the endocrine tissues naturally producing such hormones. In a particular GM salmon, for example, the hormone will occur at low levels in a year-round fashion as opposed to the seasonal expression of intrinsic growth hormone (Dunham, 2011).

Another development is the upcoming area of engineering approaches applied to biology, sometimes touted as synthetic biology, with micro-organisms and plants serving as (green) factories for pharmaceuticals and for energy (Liu, Shin, Li *et al.*, 2015; Yuan and Grotewold, 2015). It should be noted that the term "synthetic biology" has different connotations and that various international and national organizations, notably the Convention on Biological Diversity (https://bch.cbd.int/synbio/) are paying attention to this development, including its scope. In some cases, products perceived as synthetic biology could also fall under the classical definition of genetic modification so that there is substantial overlap with the GMOs described above and the regulatory and safety paradigms applied to GMOs could equally well apply to synthetic biology, on a case-by-case basis. Synthetic biology brings together systems biology, the combined metabolic network organisation of an organism, and genetic modification, the knowledge bases and set of tools that are available to molecular biologists, to obtain organisms that can be considered as user-designed organisms (Baltes and Voytas, 2015). Synthetic biology products are also envisaged to target animal nutrition, such as GM feed crops expressing synthetic proteins consisting for a great part of essential amino acids so that the protein quality of the host crop is improved [e.g. (Jiang, Ma, Xie *et al.*, 2016)]. Also if synthetic biological organisms are not developed primarily for feed materials, it can be assumed that the rest materials for any of these applications (e.g. biofuels) will become available as feeding materials.

Potential hazards

For the commercialization of GM varieties of crops and genetically modified organisms (GMOs) in more general terms, regulatory approval is needed in many countries, which entails a pre-market risk assessment according to internationally harmonized guidance of the Codex Alimentarius Commission (Codex Alimentarius, 2008). An important cornerstone of the safety assessment is the comparative approach, in which a GMO is extensively characterized and compared to a non-GM counterpart with a history of safe use (such as insect-resistant GM maize with conventional maize). Any difference thus found are then the further subject of the assessment, for which additional analyses may be warranted, such as tests for potential toxicity and allergenicity, based on what is already known of the safety profile of these compounds showing differences. This approach has thus been adopted worldwide and no confirmed safety issues have arisen over GM crops assessed and approved this way. Moreover, scientific literature on feeding trials with GM crops in livestock showed no findings of adverse impacts (EFSA GMO Panel Working Group on Animal Feeding Trials, 2008).

Various new developments in the field of plant breeding and modern biotechnology may pose challenges to the application of this approach to new products developed with newly emerging biotechnologies for various reasons, as explained below.

The section is therefore of a forward-looking nature. Based on the emergence of lower-key and more precise tools for the creation of innovative feed-producing organisms, it can be expected that there will be a wider range of modifications varying in complexity, within a broader range of host species achieved within a shorter time frame by a potentially larger group of developers. This may also lead to the creation of organisms not comparable to any used traditionally in feed production, either because of the greater impact of the modification on its host, or the fact that the host species per se has not been used substantially in feed before, which raises questions if and how the safety paradigm used so far for the safety assessment of GMOs can also be extended to these products.

Moreover, current developments show that the number of new GM plants is increasing worldwide. Contrary to the past situation in which multinational companies that had developed GM crops sought to achieve regulatory approvals globally, some GM crops recently developed by non-corporate labs in third countries are targeting only domestic markets. In such cases, developers of GMOs may not seek approvals from other countries. The latter scenario may even further enhance the risk of low-level presence (LLP) scenarios in which the adventitious admixture of trace levels of locally approved GMOs occurs in commodities shipped from an exporting country to countries where this GMO has not been filed for approval for feed purposes. These developments warrant a close following with relation to the world market (FAO, 2014).

Among future products of synthetic biology that may be incorporated in animal feed, it can be envisaged that some could be distinct from conventional feed ingredients with a history of safe use. These could include, for example synthetic nucleic acids and proteins containing non-natural building blocks, as well as new chemical metabolites. For such and other novel compounds that may pose specific chemical or biological hazards, it has to be established, on a case-by-case basis, if their safety assessment is already sufficiently safeguarded by current regulatory regimes (i.e. a knowledge gap).

Transfer to food products of animal origin

A wide range of studies on the potential transfer of genetic-modification-related proteins and DNA from GM crop-derived feed ingredients to livestock physiological fluids and tissues, as well as food products of animal origin have been extensively reviewed by the EFSA GMO Panel (EFSA, 2007) and Alexander *et al.* (2007). No indication was thus found that intact GMO-related gene and proteins would be transferred to food products of animal origin. These findings are further corroborated by the data collected from studies that have appeared since 2007 and annotated for the IPAFEED database (http://ipafeed.eu/detection-database), showing that positive detects have been reported for the digesta within the gastrointestinal tract of terrestrial livestock species but not their physiological fluids and tissues. In two studies with fish (tilapia and trout) , small fragments of a transgenic promoter DNA fragment in fish tissues occurred transiently after feeding aquafeed containing GM soybean, which probably relates to blood uptake of these small fragments and carried with the bloodstream to vascularized tissues (Chainark, Satoh, Hirono *et al.*, 2008). The preponderance of evidence thus suggests that no intact genes or proteins are transferred to food products of animal origin in a wide range of livestock species.

Human and animal health impacts

As stated above, GM feed products will have to undergo regulatory safety assessment before being commercialized and therefore there are no safety issues for these products once assessed and approved for marketing.

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NANOMATERIALS

Description of the feed products

Nanotechnology is defined by the International Organization for Standardization (ISO) as the "application of scientific knowledge to manipulate and control matter predominantly in the nanoscale … to make use of size- and structure-dependent properties and phenomena distinct from those associated with individual atoms or molecules, or extrapolation from larger sizes of the same material" (ISO, 2015). While nanoparticles, or nanomaterials consisting of such particles, are generally accepted as those with a particle size below 100 nanometres, this size limit is fairly arbitrary. There has also been debate whether concentrations of nanomaterials should be expressed on a mass basis or on a particle-number basis. In this respect, the European Commission has recently adopted a recommendation for the definition of nanomaterials, Commission Recommendation 2011/696/EU (European Commission, 2011). According to this Recommendation, a "nanomaterial" means:

Nanomaterials

Product	Manufactured nanomaterials and intentionally used purified, naturally occurring nanomaterials: Metallic and metal salt and oxide nanoparticles, such as silver, gold, zinc oxide, titanium dioxide (potential feed applications for increased bioavailability and/or e.g. unique antibacterial, coccidiostat, performance-enhancing, toxin-binding properties Polymer-based nanoparticles, including both natural (chitosan) and synthetic polymers, as vehicle (e.g. micelles) for delivery of other chemicals (e.g. feed additives) or as agent per se (e.g, pathogen-removing co-polymer of polystyrene and polyethylene glycol as feed additive) Other types of nanoparticles, such as carbon-walled nanotubes, dendrimeric polymers
Potential hazards	Physicochemical: Particles with size and surface characteristics that are conducive to uptake Inorganic particles with surface properties that favor chemical reactions leading to biomolecular and/or cellular effects Increased bioavailability of, e.g. pesticidal and therapeutic compounds, for example if residues or accidental cross-contamination cause these to be present in animal feeds and their ingredients Biological Interaction with gut microflora
Potential human and animal health impact & feed-to-animal-product transfer	Chemical, biologicaland physical hazards Inconclusive based on the scarcity of data on uptake and toxicity of nanoparticles in target livestock species , as well as the wide variety of their possible physicochemical properties
Knowledge gaps	Market penetration and commercial applications of nanomaterials in animal feeds Stability and transfer of nanoparticles from the gastrointestinal tract of livestock animals to foods of animal origin Physicochemical form, stability, and dosage of nanoparticles tested in various studies, as well as representativeness, for commercial practice, of the form of the tested nanomaterials and experimental conditions Related to the previous point, the availability of analytical methods for the purpose of characterization of nanomaterials in feed Toxicity of nanoparticles in livestock animals, such as inflammatory reactions following uptake and accumulation of particles interacting with the host immune cells, cytotoxicity, hepatotoxicity (less information than for laboratory animals) Antibacterial effects of nanomaterials present in consumed feed on the gut microflora

- A natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions are in the size range 1 nm - 100 nm.
- In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50 % may be replaced by a threshold between 1 and 50 %.
- By derogation from the above, fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm should be considered as nanomaterials.

It is expected that this definition will be used primarily to identify materials for which special provisions might apply (e.g. for risk assessment or ingredient labelling). Interestingly, the US Food and Drug Administration (FDA), under its guidance for the use of nanomaterials in animal feed, not only considers the dimensions of these materials (i.e. external dimension or an internal structure or surface within the 1-to-100-nm size range) but also whether any distinctive physical, chemical or biological characteristics can be attributed to these particular dimensions. This way, it can be clarified whether safety data are required specifically for these nanomaterials as compared to other materials with the same chemical composition (FDA, 2015).

It can be anticipated that application of nanomaterials in feed follow those in food. Therefore, an inventory of food additives and other food ingredients, food contact materials, and feed additives in the area of nanotechnologies has been composed recently for EFSA (Peters, Bouwmeester, Gottardo *et al.*, 2016). The current review builds onto that. In their review of the scientific literature, Peters *et al.* (2016) encountered various examples of direct application of nanomaterials in diverse sectors within agriculture. In addition, applications of novel nanotechnology techniques in agriculture had previously been reviewed by a number of authors (Chaudhry, Scotter, Blackburn *et al.*, 2008; Das, Saxena and Dwivedi, 2009; Gogos, Knauer and Bucheli, 2012; Kushwaha and Malik, 2012; Narayanan, Sharma and Moudgil, 2013; Shrivastava and Dash, 2012).

These applications can be grouped in four clusters:

- Nanocapsules for medicine delivery via feed (with the scenario of possible cross-contamination of conventional feeds with these nanomaterials being relevant to this review)
- Nanocapsules and nanoparticles as pesticides (while this may still be relevant as a scenario of indirect exposure of the animal via consumption of feed)
- Nanomaterials (in organic and organic form) as feed additive
- Nanoparticles for selective binding and removal of chemicals and pathogens from feed

In this review, non-persistent types of nanostructures, such as micellar aggregates of lipid molecules whose overall size falls into the nanometer range, are not considered further given that they are unlikely to sustain the various stages of gastrointestinal passage, transfer to edible tissues and other organs, and the various stages of processing in food production.

Potential cross-contamination from medicated feed

Whilst the medicinal use of nanocapsules for medicine delivery via medicated feed, including vaccines (e.g. DNA-vaccines in cultured fish) and other immuno-stimulants is outside the remit of this review, a possible scenario relevant to this work would be accidental cross-contamination of conventional feeds. A particular feature of such veterinary nanoparticles intended for oral delivery via feed is that they may have been modified so as to ensure the gastrointestinal survival and ultimately the bioavailability of bioactive compounds under the harsh conditions in the intestinal tract, such as for poly(lactic-co-glycolic acid) (Adomako, St-Hilaire, Zheng *et al.*, 2012) and chitosan nanomaterials (Khimmakthong, Kongmee, Deachamag *et al.*, 2013; Kumar, Ahmed, Parameswaran *et al.*, 2008; Kumari, Gupta, Singh *et al.*, 2013; Vimal, Abdul Majeed, Nambi *et al.*, 2014; Vimal, Majeed, Taju *et al.*, 2013).

Indirect contamination from agrochemical formulations

The formulation of pesticides and other agrochemicals (e.g. fertilizers) used on crop plants which can be used for both food and feed purposes may involve the use of nano-sized particles and micelles. This may help increase the efficacy of these agro-chemicals compared to conventional formulations through improved delivery (Frederiksen, Kristenson and Pedersen, 2003; Nguyen, Hwang, Park *et al.*, 2012; Perez-de-Luque and Rubiales, 2009; Torney, Trewyn, Lin *et al.*, 2007) and controlled release (Liu, Wen, Li *et al.*, 2006; Wanyika, Gatebe, Kioni *et al.*, 2012). Only a limited number of nano-sized agrochemical products have been commercialized (Perlatti, de Souza Bergo, das Graças *et al.*, 2012), such as Nanocid®-based pesticides (Alavi and Dehpour, 2010) and chitosan (Cota-Arriola, Cortez-Rocha, Burgos-Hernandez *et al.*, 2013). If such nanomaterials are to be commercialized as regulated pesticide products, the implications for feed safety, such as the occurrence of residues of the pesticides and the nanomaterials, are likely to be assessed before marketing under the regulatory regimes for pesticide registration, and therefore are outside the remit of this particular report.

Moreover, nanoparticles are also widely investigated as emerging environmental contaminants, as is their uptake by plants, translocation to plant tissues, and impact on plant physiology and health. To a much lesser extent has the translocation and accumulation of such nanoparticles, such as carbon-based fullerenes, into edible parts of food and feed crops been studied [e.g. reviewed by Pacheco and Buzea (2017)].

Nanomaterials as feed additive

There is a growing number of scientific papers that report on the possible use of feed additives containing nanomaterials. The claimed functions are diverse ranging from potential growth enhances, application to improve the appearance of the end product or the removal of pathogens or chemical toxicants like mycotoxins.

A range of nanomaterials is studied as means to enhance the growth performance of food producing animals (see Table 9). The mechanism behind the growth enhancement is not known and (at least for some nanomaterials) it is speculated to include an antimicrobial effect either against certain bacterial groups or reducing the microbial load of the small intestine (Fondevila, Herrer, Casallas *et al.*, 2009). Other beneficial effects over the host metabolism were also not discarded by these authors. Nanoparticles consisting of polymers, including amphipathic block co-polymers

Table 9: Use of inorganic or organic nanomaterials claimed to enhance the growth of food producing animals

Chemical composition of inorganic nanomaterials	Food-producing species	Investigated production parameters	References
Silver	Weaned piglets	Performance	(Fondevila *et al.*, 2009)
Silver	Broiler chicks	Performance, animal nutrition, animal health, mycotoxin toxicity mitigation	(Ahmadi, 2012, Gholami-Ahangaran and Zia-Jahromi, 2013)
Zinc oxide	Broiler chicks	Performance, animal nutrition, animal health	(Ahmadi, Ebrahimnezhad, Sis *et al.*, 2013; Zhao, Tan, Xiao *et al.*, 2014)
Zinc oxide	Freshwater prawn (Macrobrachium rosenbergii)	Performance, animal nutrition, animal health	(Muralisankar, Bhavan, Radhakrishnan *et al.*, 2014)
Chromium	Pig (finishing gilts)	Performance, animal nutrition, animal health, carcass characteristics	(Hung, Leury, Sabin *et al.*, 2014; Sales and Jancik, 2011)
Chromium nanocomposite	Pig	Animal nutrition	(Wang, Li, He *et al.*, 2012b)
Selenium	Layer chicks	Performance, animal nutrition, animal health	(Mohapatra, Swain, Mishra *et al.*, 2014)
Selenium	Sheep	Animal nutrition	(Xun, Shi, Yue *et al.*, 2012)
Iron	Poultry	Performance, animal nutrition, animal health, food product characteristics	(Nikonov, Folmanis, Folmanis *et al.*, 2011)
Use of organic nanomaterials	**Food-producing species**	**Investigated production parameters**	**References**
Dietary mixture of Aspergillus probiotic and selenium nano-particles	broiler chickens	Performance, animal nutrition, animal health, food product characteristics	(Saleh, 2014)
0.4% turmeric extract nanocapsule	broiler chicks	Performance, animal nutrition, animal health, food product characteristics	(Sundari, Zuprizal, Yuwanta *et al.*, 2014)
Chitosan	Tilapia (Oreochromis nilotica)	Performance, food product characteristics	(Wang and Li, 2011)
Chromium-loaded chitosan nanoparticles	Pig (finishing gilts)	Performance, animal nutrition, animal health, food product characteristics	(Wang, Wang, Li *et al.*, 2012c; Wang, Wang, Du *et al.*, 2014)
Copper-loaded chitosan nanoparticles	weaned piglets	Performance, animal nutrition, animal health,	(Wang *et al.*, 2012a)
Copper-loaded chitosan nanoparticles	broilers	Performance, animal health	(Wang, Wang, Ye *et al.*, 2011)
Silicon nanomaterials as carrier of nutrients		Animal nutrition	(Canham, 2007)

a performance includes measures such as body weight gain, feed intake, animal food product yield of polystyrene with polyethylene glycol, have been described as candidate feed additives with the purpose of removing pathogens from the intestinal contents [e.g. (FAO/WHO, 2010, Kuzma, 2010)]. Moreover, derivatized, cationic nanoscale polymers may also exhibit true antibacterial effects themselves, whilst a range of studies have focused on nanosilver given the known antibacterial action of silver per se (Hill and Li, 2017). The antibacterial effect of nanoparticles has also been studied in various studies for its impact on the rumen and gut microflora, given the impact of this flora on animal performance and health [e.g. (Wang, Du, Wang *et al.*, 2012a)]. Further research is warranted to address this knowledge gap of antibacterial effects of nanoparticles on livestock gut microflora. Interestingly, for nanomaterials consisting of zinc oxide, the various beneficial health impacts in experimental livestock studies, including growth enhancement and immunomodulation were achieved at lower administration rates than conventional zinc oxide agents, whilst the nanoparticles also exhibited antibacterial actions, such as against mastitis in dairy cattle (Swain, Rao, Rajendran *et al.*, 2016).

As mentioned, the main aim of the incorporation of (in) organic nanomaterials in feed is to enhance the growth performance of food producing animals. In addition to that, one clear example was found of a study in which a nanomaterial was included in chicken feed with the sole purpose to modify the appearance of a product. In this case a micro-emulsified pigment consisting of carotenoids was used to create the desired yolk colour of chicken eggs (Chow, Gue, Leow *et al.*, 2014).

Nanoparticles for selective binding and removal of chemicals and pathogens from feed

The last group of applications is the use of nanomaterials for the removal of pathogens or chemical toxicants from animal feed. Examples are a nano-sized additive based on montmorillonite clay (nanoclay) that is used to bind mycotoxins that may be present in the animal feed (Shi, Xu, Feng *et al.*, 2005) and magnetic nanomaterials for inactivating two mycotoxins, aflatoxin B1 (AFB1) and zearalenone (ZEA) in feed (Kim, Kim, Lee *et al.*, 2012). For this application critical questions on the intestinal fate of the nanomaterial-toxic complexes can be raised. For example, the processes during intestinal digestion of these complexes is not (or only limited) studied. Potentially the previously bound toxicants are released again from the nanomaterials during stomach or intestinal transit. No studies on this could be found in the literature.

Potential hazards

Given that agencies such as FDA are not aware of any engineered nanomaterials that can be declared as generally recognized as safe for use in animal feed (FDA, 2015), there is no practical experience as yet with the regulatory risk assessment and commercialization of such products. Several EU member states have registries for nanomaterials where companies have to notify such materials and products containing them if produced and/or merchandized by these companies. In the French R-Nano register's annual summaries of materials notified during a particular year, animal feeds are also reported as a target for the use of some nanomaterials such as silicon dioxide (ANSES, 2018). Yet, we are unaware of regulatory risk assessments of these materials for feed use. Moreover, both the US and European experiences show that there is still a lack of a comprehensive inventory of the types and volumes of engineered nanomaterials being used for animal feed purposes.

Interestingly, EFSA's Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) has considered the possibility that products of iron oxide, which has a history of use as feed additive, may partly consist of particles within the nano-size range (EFSA, 2016a; EFSA, 2016b). With regard to transfer from feed to food products of animal origin, this Panel concluded that this was unlikely to happen for ferric oxide. Yet due to the method used for size distribution, particularly in older dossiers, the nature and share of the iron oxide nanoparticles in the product remained unknown (EFSA, 2016a, EFSA, 2016b). A similar reasoning can also be applied to other feed additives, such as titanium dioxide. Whilst other expert Panels active within EFSA have already assessed nanoparticles for their use in food additives and food contact materials (i.e. packaging), FEEDAP was unable to conclude on these nano-sized fractions due to lack of data on prevalence and size distribution, among others. In draft guidance recently posted on the Internet for comments, the EFSA FEEDAP Panel recommends that, in case of the likely occurrence of nanoparticles within a feed additive, the size distribution of particles is to be determined through laser diffraction analysis (EFSA, 2017).

As a consequence of their small size, nanomaterials can exhibit different physicochemical properties and biological effects compared to their respective bulk materials, even at the same mass dose (Oberdorster, Oberdorster and Oberdorster, 2005). Up to now only for a few nanoparticles a risk assessment is available in the scientific literature, perhaps the best described cases are for silica and silver nanomaterials (Dekkers, Bouwmeester, Bos *et al.*, 2013; Dekkers, Krystek, Peters *et al.*, 2011; Wijnhoven, Peijnenburg, Herberts *et al.*, 2009). But even these assessments are hampered by uncertainties, mainly due to the lack of reliable characterization data of the nanomaterial in the product, and inadequate material characterization in the toxicological studies performed. Important also in this regard is to ensure that the nanomaterials tested in the various in-vitro and in-vivo models for toxicity have retained the same characteristics as the materials under real-life conditions of commercial use. The OECD (2012) has established a companion guidance for the characterization and dosage measurement of nanomaterials used in toxicological assays. In this guidance, various factors that could affect the physicochemical state of the materials, such as preparation method (e.g. duration of sonication), storage stability, as well as ionic strength, pH, and purity of the solution, are listed as parameters to consider in the design, performance, and reporting of studies (OECD, 2012). Interestingly, Bergin *et al.* (2013) note that the actual nature of the nanomaterials may change during passage through the gastrointestinal tract, such as the formation of a "corona" of molecules absorbed to the nanoparticles, which may either reduce or aggravate toxicity of the particles (Bergin and Witzmann, 2013). Also the aggregation of nanoparticles at higher concentrations within dosed preparations may account for the higher toxicity observed at relatively low dosage levels, for example (Bergin and Witzmann, 2013). There is therefore a knowledge gap given that there is a lack of details on the physicochemical properties, stability and behaviour of nanoparticles used in a number of animal studies. This also holds true for data on the availability of analytical methods to test for these parameters.

Amongst the various types of toxicity reported for nanoparticles entering the animal or human body via the gastrointestinal tract, there are a number that report inflammatory reactions (similar to lung-inhaled nanoparticles), hepatotoxicity, oxidative stress, and others. These reports are, however, scarce and may not always be consistent depending on the chemical form, formulation and dose levels, and animal model investigated [e.g. (Bergin and Witzmann, 2013; Swain *et al.*, 2016)]. Moreover, these is a knowledge gap in that such studies are usually performed in laboratory animal species and not livestock target species.

In conclusion, the incorporation of nanomaterials in animal feed is studied at a scientific level, with a potentially wide range of nanomaterials under investigation. It is however not clear if sufficient safety data have accumulated for these nanomaterials, so as to ensure that they can be safely applied in feed and if they are also commercialized for feed purposes.

Transfer to food of animal origin

The field of nanotoxicity developed and matured in the past decade, but much needs to be revealed. The risk assessment of nanomaterials still heavily relies on animal studies (Bouwmeester, Brandhoff, Marvin *et al.*, 2014). For the human safety assessment rodent species are used and no or only very few food-producing animals have been used in toxicological studies. A further complicating factor is that, from a toxicological point of view, potential edible parts of animals, like muscles are not routinely assessed in toxicokinetic studies. In conclusion no, or very limited information is available on the potential transfer of nanomaterials from animal feed to edible tissues, milk and eggs, indicative of a knowledge gap. It is out of scope of this report to discuss the potential transfer and associated hazards of each nanomaterial individually.

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New developments in analytical methods for the detection of hazards

In this chapter new developments in analytical methods for the detection of hazards are described. A subdivision is made between methods of analysis for chemical, biological and physical hazards. The aim of this chapter is to give a general overview of methods of analysis that can be applied for the detection and quantification of potential hazards in feed and feed ingredients and in this way give guidance to stakeholders and in particular analysts concerned with sample analysis. In the framework of this background document it was not possible to make a comprehensive overview that includes methods for all potential hazards, e.g. for all (emerging) plant toxins and all bacterial agents.

It should also be noted that the choice of the analytical method, the frequency of sample analysis and the location where lots are sampled depend on the risk management measures that are in place.

CHEMICAL HAZARDS

The aim of this section is to give a general overview of methods of analysis that can be applied for the detection and quantification of chemical hazards in feed and feed ingredients and to highlight new developments since 2007.

The choice of the method of analysis for chemical hazards depends on the type of compounds with on the one side organic compounds and on the other potentially toxic elements (often referred to as heavy metals).

Organic hazards

Nowadays, for organic hazardous compound chromatographic methods are most frequently used. For polar (water-soluble) and medium-polar compounds, such as mycotoxins, plant toxins and veterinary drugs, high-performance liquid chromatography (HPLC) is the method of choice while for apolar (lipid-soluble) compounds, such as dioxins, PCBs and organochlorine compounds, gas chromatography (GC) is applied.

Since the 2007 FAO/WHO Expert Meeting on Animal Feed Impact on Food Safety (FAO, WHO, 2008b) the following trends can be observed:

- Mass spectrometry (MS)-based detection methods have replaced the formerly applied HPLC-ultraviolet (LC-UV) and HPLC-fluorescence methods and the GC-flame ionization detection (GC-FID) and GC-electron capture detection (GC-ECD) methods for many applications. The main reasons for this shift are (i) that with mass-spectrometric methods a better sensitivity can be obtained, which means that lower levels of the compounds can be detected and (ii) that this detection mode allows to confirm the identity of the compounds, which is important, among others, in official control (Hird *et al.*, 2014). Hyphenated MS-techniques have extended their applicability by integrating approaches to resolve important drawbacks such as loss or enhancement of signal due to matrix effects by incorporating stable isotope dilution and normalization strategies to account for the impact of the vast array of matrices that enter in the composition of food and feed (Jackson et al, 2012; Zhang et al, 2014).

- Shift from single-analyte methods, where only one hazardous compound can be determined, to multi-analyte and multi-class methods (so called multi-methods) that allow the determination of a whole class or even several classes of compounds in one analytical run (van der Lee *et al.*, 2008; Alwis & Heller 2010; Boscher *et al.*, 2010; Nardelli *et al.*, 2010; Kaklamanos *et al.*, 2013; Kalachova *et al.*, 2013; Krska & Nielen, 2013; Lankova *et al.*, 2013; Tolosa *et al.*, 2014; Tsiplakou *et al.*, 2014). Obviously, the main advantage is that the costs of analysis will be reduced significantly. Moreover, samples can be tested for different classes of compounds and this may lead to new insights regarding the presence of certain classes of compounds.
- Many different types of MS-detectors are available and new types are still entering the market. For GC, high-resolution MS detectors are already used for many years, especially for the determination of dioxins and dioxin-like PCBs (FAO, WHO, 2008b). Nowadays, for LC-MS high-resolution (HR) MS-detectors are also available. The benefits provided by HRMS techniques include the collection of full-scan spectra, which provides greater insight into the composition of a sample. Consequently, the analyst has the freedom to measure compounds without previous compound-specific tuning and the possibility of retrospective data analysis, which means that the spectral information can be investigated again at a later stage when new hazardous compounds have been discovered to see if these hazardous compounds were present in the sample. Furthermore, LC-HRMS techniques have the capability of performing structural elucidations of unknown or suspected compounds. HRMS is one of the most promising tools when moving towards non-targeted approaches (Kaufmann 2012).

Potentially toxic elements

For potentially toxic elements (arsenic, cadmium, lead and mercury) most laboratories apply atomic absorption spectroscopy (AAS) in different formats for the various compounds. Single-analyte methods for compound feed, feed materials, premixtures and feed additives are well established and standardized. Multi-methods based on inductively coupled plasma (ICP) - atomic emission spectrometry (ICP-AES) that are primarily focused on minerals and trace elements, may also be applied for lead and cadmium, but only for higher levels in mineral products (EN 15621:2012, via internet link 1). Since 2007 the trend is towards the use of ICP-MS multi-methods where potentially toxic elements can be determined together with minerals and trace elements.

As described in section 3.1.2, inorganic arsenic is much more toxic than organic arsenic and methylmercury is much more toxic than inorganic mercury. For that reason it is important to differentiate between these different forms through so-called speciation analysis. Several methods have become available for e.g. fish feed based on HPLC-ICP-MS, which allow the determination of inorganic arsenic and methylmercury (Sloth *et al.*, 2005; Vallant *et al.*, 2007; Hedegaard & Sloth 2011). For inorganic arsenic, a method is also available where inorganic arsenic is first separated off-line by means of solid-phase extraction, allowing to perform the determination with the standard AAS-method (Rasmussen *et al.*, 2012). The method has been standardized by CEN (EN 16278:2012, via internet link 1).

Screening methods

The instrumental methods described above, that can be regarded as “golden standards” are relatively expensive and require well-trained staff in sophisticated laboratories. For many hazardous compounds screening methods have been developed that are less costly, easier to perform and do not require well-equipped laboratories. In many cases, these screening methods are based on the bio-molecular interaction between a hazardous compound and a specific antibody or other types of biomolecules (e.g. receptors, aptamers). Different platforms have been developed to measure this interaction: ELISA-plate screening methods for mycotoxins and veterinary drugs are commercially available ((Jimenez *et al.*, 2010; Rai *et al.*, 2011), these methods can be executed in laboratories with simple equipment. Dipstick tests (or so-called lateral flow devices) have also been developed (Kolosova *et al.*, 2008). The advantage of these dipsticks is that they can also be used under field conditions in small feed mills and ports when staff is available that has been trained. Another advantage is that the results of the test are available within some hours. The trend since 2007 is that multi-screening methods become available, e.g. multi-dipstick methods for Fusarium mycotoxins (Lattanzio *et al.*, 2013) and tropane alkaloids (Mulder *et al.*, 2014) and a multi-ELISA method for pyrrolizidine alkaloids in feed (Oplatowska *et al.*, 2014). Recently, a special issue of the World Mycotoxin Journal has been dedicated to rapid methods for mycotoxin detection (World Mycotoxin Journal, 2014). Since 2007 several instrumental platforms for bio-molecular methods have been developed, e.g. surface plasmon resonance (SPR) for multiplex microassay sensing of mycotoxins (Dorokhin *et al.*, 2011), multiplex flow-through immunoassay formats for screening of mycotoxins (Ediage *et al.*, 2012), multiplex flow-cytometry with bead technology for mycotoxins (Peters *et al.*, 2013) and a microsphere immunoassay with imaging planar array detection for mycotoxins (Peters *et al.*, 2014). These instrumental platforms can be coupled to autosamplers and thus lend themselves to high sample throughput which is an advantage if many samples have to be analysed. Some of these instruments are becoming available as portable instruments with sufficient robustness for field applications. Biosensor-based methods for antimicrobial residues in food have been reviewed (Huet *et al.*, 2010).

For the determination of dioxins and dl-PCBs, cell-based bio-assays such as the Calux-assay can be applied. These bio-assays are successfully applied in various laboratories for screening purposes. Since 2007 no new developments were reported.

Near-infrared (NIR) hyperspectral imaging is a technique which allows via a camera the measurement of spectra from single particles in feed materials. By interpretation of the obtained spectra in an automatized manner, botanical impurities such as ergot bodies can be identified (Vermeulen *et al.*, 2013). While this technique does not allow the direct detection of chemical hazards, through the botanical impurities the chemical hazards can be detected in an indirect way.

For the screening of the mycotoxin deoxynivalenol (DON), the application of a so-called electronic nose, based on metal oxide sensors, has been described (Campagnoli *et al.*, 2011; Lipollis *et al.*, 2014). This method does not detect DON itself but is based on indirect detection of volatile products of fungal metabolism. So far, the method was only tested for durum wheat for food applications.

Another screening method for DON in durum wheat is based on Fourier Transform (FT)-NIR (De Girolamo, 2014). With a cut-off value of 1.400 µg/kg, the method could be successfully applied to durum wheat. FT-NIR and dispersive NIRS techniques were also described for the screening of aflatoxin B1 (AFB1) in maize and barley (Fernández-Ibañez *et al.*, 2009). According to these authors, because AFB1 occurs in small concentrations, it is not likely that NIRS can detect AFB1 directly. However, contamination by aflatoxins affects other chemical and optical properties of whole kernels that can be detected with NIR spectroscopy. A major drawback of FT-NIR is the high dependence on the product characteristics and the consequent need for appropriate calibration. The opportunities for application of these techniques to routine analysis therefore presuppose expansion of calibration databases (Lattanzio *et al.*, 2009).

Screening methods are typically applied in situations, where (i) a high number of samples need to be analysed and checked against a target level (often the maximum limit) of an analyte and (ii) it can be reasonably assumed that the majority of samples are below this target level. Screening tests are designed to identify samples exceeding this level. Negative samples are accepted as such, but positive results need to be re-analysed by confirmatory methods. The application of screening tests may lead to false positive results. The percentage of false positive results should be low. Even more important is that the percentage of false negative results is low because in this case non-compliant products will enter the feed chain. Often a maximum of 5 % false negative results is applied.

Standardisation

Methods or analysis may differ largely in their degree of validation. Some methods have only been validated in the laboratory where the method was developed. Other methods have been validated by means of international collaborative studies and consequently evidence is obtained that the method can be transferred to other laboratories.

Several international organizations are involved in the preparation and publication of standardized methods for chemical and biological hazards in feed. Worldwide AOAC International develops methods that are published as Official Methods (AOAC, 2012). Among others, methods for antibiotics are included (see internet link 2 for further information).

In Europe, the CEN (European Committee for Standardization) committee TC 327 "Animal feedingstuffs - methods of sampling and analysis" has standardised / is currently standardising methods based on international collaborative studies for among others mycotoxins, dioxins and DL-PCBs, NDL-PCBs, potentially toxic elements (including speciation), organochlorine pesticides, plant alkaloids, feed additives and antimicrobial growth promoters (see internet link 1). The trend is towards the use of multi-methods, in many cases based on LC-MS/MS (organic compounds) or ICP-MS (inorganic compounds).

The criteria approach that was developed by the Codex Committee on Methods of Analysis and Sampling (CCMAS) as an alternative to method standardization (FAO, WHO, 2008b) is currently in the work programme of CEN /TC 327, where criteria are under development for mycotoxins and potentially toxic elements.

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Internet links

1. CEN TC 327 : http://standards.cen.eu/dyn/www/f?p=204:7:0::::FSP_ORG_ID:6308&cs=151B54DCC1DA2676999693FCE3A6F61BC
2. http://www.aoac.org/iMIS15_Prod/AOAC

BIOLOGICAL HAZARDS

Microbiological hazards

Okelo and Fink-Gremmels (2012) have reviewed methods for pathogen detection and microbial enumeration techniques relevant for control measures that can improve animal and human safety during production, storage, distribution, and use of animal feed. They indicate that industry recognizes pathogenic bacteria like *Salmonella* spp. as attributing to food-borne cases, yet also identifies other bacteria like Bacillus spp., Listeria, pathogenic Escherichia coli, and spore-forming clostridia as contaminating grain, feed ingredients, and animal feed (Okelo and Fink-Gremmels, 2012). In this section, a general overview of microbiological analyses and some background on conventionally applied methods are presented. Furthermore, information on newer analytical methods for the detection of microbiological hazards, especially *Salmonella* spp., are elucidated.

Microbiological analyses include detection methods (e.g. mainly qualitative methods) and enumeration methods (e.g. quantitative methods) of micro-organisms. Qualitative methods indicate the presence or absence of an organism, while quantitative methods attempt to enumerate micro-organisms in food or feed either directly (e.g. bacterial colony counts) or indirectly (e.g. measuring certain parameters of target micro-organisms in the growth media) (Okelo and Fink-Gremmels, 2012). Alali,Ricke and Fink-Gremmels (2012) have emphasized that improvements in programs that monitor feed production as well as the use of sensitive and rapid pathogen detection methods are required to reduce the incidence of pathogens in the feed.

Conventional analytical methods

Culture methods such as detection in broth and agar media as well as the use of most probably number (MPN) methods have been conventionally employed to detect or enumerate microbiological hazards. Molecular methods such as polymerase chain reaction (PCR) also have been extensively used as they offer several advantages. Nevertheless, these methods still have some difficulties (e.g. in-distinguishable viable and non-viable counts), yet these can often be corrected with a pre-enrichment step before PCR analyses (Okelo and Fink-Gremmels, 2012). Despite these conventional methods, newer analytical techniques for rapid microbiological hazard detection in food and feed are further elaborated.

Newer analytical techniques

Okelo and Fink-Gremmels (2012) have outlined some emerging technologies for detection and enumeration of microbiological hazards including the use of chromogenic and fluorogenic growth media, yet also more rapid bacteriophage-based and impedance-based techniques. Nonetheless, a need for rapid and accurate detection in parallel remains. For example, Suh,Jaykus,Brehm-Stecher *et al.* (2013) emphasized that despite newer, rapid detection techniques of foodborne pathogens, some methods remain insensitive and can generate matrix related inhibitory compounds. Alternatively, these authors have suggested utilizing pre-analytical sample treatments with target-specific bioaffinity ligands that can prevent co-precipitation of target pathogens with residual matrix components (Suh *et al.*, 2013). In particular, Suh *et al.* (2013) have investigated bioaffinity ligands such as bacteriophages, phage-derived biomolecules, nucleic acid/peptide aptamers, carbohydrate ligands, antimicrobial peptides, and synthetic ligands.

While reviewing new pathogens in microbial detection, Kahyaoglu,Irudayaraj and Sofos (2013) have also emphasized the need for the standardization and development of new, sensitive methods, yet which concern virus detection. Immunological and PCR-based methods are commonly employed for virus detection in foodstuffs, yet unfortunately, are limited regarding speed and sensitivity. Kahyaoglu *et al.* (2013) have indicated that electrochemical-based detection techniques, spectroscopic, and microfluidics assays, among others, are expected to become more employed in the coming years.

Concerning molecular techniques, omic technologies including genomics, proteomics, and metabolomics are currently being used to investigate pathogen behaviour at the molecular level alongside improvements in pathogen detection and typing. Such technologies research biological processes in a quantitative and integrative way. However, challenges include the implementation of genomic and proteomic studies in food and complex matrices, e.g. animal feed, alongside interpretation and analysis of these results (Fratamico, Gunther IV and Sofos, 2013).

Salmonella spp. detection

For microbiological hazards like *Salmonella* spp. in animal feed, the loop-mediated isothermal amplification (LAMP) method has been identified as a promising development concerning specific pathogen detection. Kokkinos, Ziros, Bellou *et al.* (2014) have reviewed LAMP application for *Salmonella* spp. detection in food matrices in comparison to conventional culture techniques. In short, LAMP-based methods appear to be robust and innovative molecular diagnostic methods for use in the food and agricultural industries as well as by public health authorities. For *Salmonella* spp. in seafood, Amagliani, Brandi and Schiavano (2012) have noted rapid methods such as membrane filtration, automated electrical techniques, and immunological assays as providing effective alternatives.

Amagliani *et al.* (2012) emphasized that PCR and Real-Time PCR methods remain the most promising due to high sensitivity and selectivity. Moreover, Rantsiou, Cocolin and Sofos (2013) have elaborated on the developments within PCR analysis emphasizing that quantitative PCR (qPCR) has transformed molecular approaches due to its ability to quantify specific pathogens in food. Nevertheless, quantification of *Salmonella* in many (dry) feed materials is challenging due to the heterogeneous distribution of the bacteria and to the presence of living, but unculturable *Salmonella* in the in the harsh environment. Differences in kinetics of pre-enrichment and real-time PCR are likely to introduce bias in quantification (Schelin, Andersson, Vigre *et al.*, 2014).

DNA microarrays can provide a new method for the transcription process to allow one to investigate the behaviour of pathogens in food environments. Jarquin,Hanning,Ahn *et al.* (2009) have reviewed the development of rapid detection and genetic characterization of *Salmonella* in poultry breeder feeds and indicates that PCR assays and DNA array technologies have been used to disseminate specific *Salmonella* serotypes (e.g. at feed mills). Overall, pathogen detection systems such as bead-based DNA microarray coupled with flow cytometry and PCR amplification was concluded as an advantageous methodology due to its simplicity, reusability, multiplexing capabilities, cost-effectiveness, sensitivity, and practically for other feed types and feed ingredients (Jarquin *et al.*, 2009). However, the reliability of microarray typing for *Salmonella* in feed materials may be interfered with by DNA from bacteria in the high background flora (102–107/g) present in some feed materials which has been suspected to react non-specifically with some of the typing probes (Koyuncu, Andersson, Vos *et al.*, 2011). Besides these methods, Nourmohamadi and Shokrollahi (2014) have communicated the possibilities for multiplex polymerase chain reaction (M-PCR) methods as yet another newer alternative analytical method for detecting poultry feedstuff contamination by *Salmonella* serotypes.

While many novel detection methods have been published for the detection of *Salmonella* in “food and feed” the sensitivity is in practice often limited by poor pre-enrichment in the broth used. For example, the acidic nature of many dry feed materials may result in failure of *Salmonella* to reach detectable levels during pre-enrichment due to low pH in broth (Cox, Cason, Buhr *et al.*, 2013).

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GMOs and synthetic biology

There is a growing number of GM crops worldwide that are brought to the market (James, 2016). For their use in food and feed, pre-market approval is needed from national authorities under the law of many nations. This legal requirement entails a regulatory application procedure. A common part of this procedure is an extensive safety assessment of the use of these crops for food and feed purposes, ensuring that what comes to market is no less safe than conventional crops.

Upstream of the regulatory testing and application procedures, a wider array of experimental GM crops may actually be tested in laboratories, greenhouses and in the field, some of which may make it to the next stage of commercialization, whilst some others may not. Moreover, various edible GM crops have been developed for the production of non-food/feed products. For both categories of crops, regulatory bio-containment requirements are in place to prevent any mix-up with crops grown for food and feed purposes. Examples of events leading to such mix-ups are unwanted cross-pollination in the field, volunteer plant formation in follow-up crop rotations, and other forms of accidental commingling.

Despite these preventive measures, there have been a number of incidents of unwanted admixture of non-approved GM crop varieties into mainstream food and feed supplies. Although several historic cases have also led to tightened regulations and enforcement [e.g. (Kleter, Prandini, Filippi *et al.*, 2009)], finds of adventitiously present unapproved GM crops continue to happen. This is exemplified by the continuing finds of unauthorised genetically modified materials being reported through the European Commission's Rapid Alert System for Food and Feed (RASFF) (European Commission, 2018). Increasingly, unauthorised GMOs are identified that have not been approved anywhere in the world. A plausible source in many cases is accidental cross-contamination during the breeding stages with experimental or non-food/feed GM crops for which the food & feed safety has not or not yet been assessed (FAO, 2014). Given that their food and feed safety may not have been assessed yet, they constitute a safety hazard.

These incidents involving unapproved GM crops also highlight the importance of detection methods for identification and verification purposes at the different stages of food and feed production, ranging from sowing seed to the final product for consumption. Given the ever increasing number of field-tested and other unapproved GM crops that may in theory lead to mixed seed batches in the respective crops, initial screening will become more and more demanding to cover the broad range of genetic modifications. In this respect it is also increasingly relevant to focus on those GMOs that may be most relevant in terms of feed safety in addition to food safety and the environment, in order to use the available analytical capacity as effectively as possible. For specific GMOs on-site methods have been developed, such as dipstick methods (Wang, Teng, Guan *et al.*, 2013), but these generally will not be helpful for broader screening purposes. Other on-site methods are being developed (http://www.decathlon.eu) and may in the future become applicable when screening programmes may focus on specific GMOs.

For GMOs, currently the standard method is DNA-based detection, which can be highly specific for a given GM crop variety or more broadly focused on multiple GM crops sharing a common introduced DNA element. Such DNA-based detection commonly relies on real-time or quantitative polymerase chain reaction (qPCR) methods. For GMOs that are in the pipeline for approval for the European market, it is, for example, obligatory for the applicant to supply a GMO-specific polymerase chain reaction (PCR) method, for other countries this will often not be the case. A GMO-specific method will generally identify the sequences that bridge the newly introduced genetic construct and the endogenous plant DNA. For unauthorised GMOs that have not (yet) been submitted for market approval in a particular country, and thus have not yet been assessed for their food, feed or environmental safety in this country, such GMO-specific methods will in general not be available. Even if the GMO has been assessed anywhere else in the world, this does not mean that a specific method has been required, or even that on the basis of the available information on a particular GMO, a method can be developed. To overcome these limitations, for the detection of unauthorised GMOs nowadays a screening approach is applied that makes use of methods for a range of different GMO-related DNA elements. Based on the outcome of this initial screening step, it can be determined which approved or well-characterised unapproved GMOs may be present in a sample and in the next confirmation step available methods for these GMOs can be applied. Should, on the basis of this confirmation step, all elements from the first round be explained by the detected GMOs, then it is assumed that no additional GMOs will be present in the sample. However, if not all GMO elements that have been detected can be explained by the detected GMOs, then these GMO elements may indicate the possible presence of additional unauthorised GMOs. In these cases, the subsequent step will be to sequence the sample starting from the respective elements and try to identify in this way any unauthorised GMO that may be present in the sample (ENGL ad hoc working group on “unauthorised GMOs”, 2011; Prins, van Dijk, Beenen *et al.*, 2008; Scholtens, Laurensse, Molenaar *et al.*, 2013).

In order to improve the cost effectiveness of analytical procedures there are currently a number of different developments. One of the bottlenecks that is currently observed in the identification and quantification of GMOs in especially more complex matrices, is that there may be PCR-inhibiting factors available in the matrix that will hamper the identification and quantification of the GMOs in the sample. To overcome these difficulties the added value of digital droplet PCR is being explored. This PCR procedure occurs in an emulsion PCR where ideally one template is present in an individual droplet, leading to optimal PCR conditions and thus an improved sensitivity also in more complex matrices (Morisset, Stebih, Milavec *et al.*, 2013).

Another interesting and theoretically more powerful development is the upcoming use of DNA sequencing methodologies to sequence either DNA as isolated from individual samples on the basis of whole genome sequencing (WGS), or to sequence DNA as isolated from individual samples after a specific enrichment step for GMO-related sequences. In the latter case there are basically two options: either the GMO-related sequences are enriched for in a PCR step, leading to essentially the same information as compared to the screening step in the PCR procedure, but allowing a fully automated data-analysis. Or, in a more advanced approach, not only the known sequences are amplified, but also the adjacent unknown sequences, allowing for screening and confirmation of the GMOs in a single assay. For this development to become practically feasible it will be necessary to develop a detailed protocol based on the specific characteristics of individual sequencing platforms in combination with effective procedures for subsequent data analysis. Complicating factors are here the different procedures for amplification and labelling prior to the sequencing step, as well as the many different protocols leading to different read lengths of the resulting amplified DNA fragments (Liang, van Dijk, Scholtens *et al.*, 2014).

Both approaches are currently being explored in different national as well international research projects and so far, the results are encouraging. It is likely that especially the screening protocols will become more effective in the years to come, whilst for more effective confirmation protocols it may also be necessary to obtain detailed sequencing data related to GMOs rather than a GMO-specific detection method.

A final observation is that, with the advent of synthetic biology, also the sequencing strategy may need to be timely adapted, should it become more feasible to use also synthetic nucleotides with other basic characteristics. But also in this respect it seems most relevant to stay well informed on the global developments in terms of research in the area of genetic modification and synthetic biology as that seems the best key to adequate methods for detection and identification of raw materials that may affect the safety of animal, man or the environment.

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Animal proteins

The strategy to eradicate Bovine Spongiform Encephalopathy (BSE) and related prion diseases from the feed production chain is to control for the absence of certain processed animal proteins (PAPs) in selected feed types.

As described in section 3.2.5, restrictions on the use of PAPs in feed vary from country to country, based on their own scientific risk assessments. The minimum ban recommended by the World Organisation for Animal Health (OIE) is on feeding ruminant by-products to ruminants with some exceptions, incl. milk. As a consequence of the differences in the restrictions, the need for methods of analysis also differs from country to country. Obviously, the need for methods was the highest in Europe after the major outbreak after 1988. For that reason, in this section the restrictions that were put in place in Europe will be outlined as a worst case scenario to give context to the set of methods of analysis that may be required.

The European Union ban is based on the principle that PAPs are not fed to ruminants (ruminant ban), there is a range of exceptions, e.g. weaning calves are allowed to consume fish meal as milk replacer, and milk and milk products are allowed for all farmed animals. Comparable versions of the ruminant feed ban are enforced in USA, Japan and China (Liu *et al.*, 2011). Currently an extended feed ban is in force in the EU restricting the feeding of PAPs of terrestrial animals to terrestrial animals; feeding to fish is allowed. The exceptions have the consequence that enforcement scenarios will be complicated.

The tool box of inspection methods at the time of the FAO report in 2007 was described by van Raamsdonk *et al.* (2007). The major methods of analysis that are used to control for the absence of processed animal proteins are optical microscopy, DNA-based methods (Polymerase Chain Reaction, PCR) and immuoassays, whereas minor shares are provided by near infrared (NIR) spectroscopy and NIR microscopy, mass spectrometry and fatty acid profiling.

Current analytical methods

The current situation focuses on detection of PAP by microscopy in most cases and PCR for identification. Immunoassays are available for specific cases.

Classical light microscopy

The light microscopy method is capable of distinguishing between fish material and land animal material at a level below 0.005% (w/w) in feed (Veys *et al.*, 2010). The primary target consists of the bone fragments, which are usually present in any processed animal protein. Besides this, muscle fibres, hair fragments, feather filaments, fish bones, cartilage and in special circumstances also blood meal can be detected (Fumière *et al.*, 2009; van Raamsdonk *et al.*, 2011).

The combined use of microscopic methods in association with computer image analysis to identify the origin of PAP appeared promising, especially as a complementary method for the DNA-based methods (Pinotti et a., 2013). While multivariate analysis is a helpful tool for microscopic discrimination between mammalian and avian bone particles, still only first indications can be given with respect to the origin and nature of the encountered materials in samples from practice (van Raamsdonk *et al.*, 2012b).

PCR

A range of species can be identified using specific primer-probe combinations (Fumière *et al.*, 2006; Cawthraw *et al.*, 2009; Rojas *et al.*, 2011; Pegels *et al.*, 2011; Kesmen *et al.*, 2013; Pegels *et al.*, 2014a, 2014b). The official method in the EU is targeted on ruminant material (Fumière *et al.*, 2012; EURL-AP, 2017). This method has been validated for animal feed and processed animal proteins in interlaboratory studies by the European network of National Reference Laboratories (Fumière *et al.*, 2016; Olsvik *et al.*, 2017). Sensitivity depends on the specific circumstances and can range from 0.001% to 0.1%. A drawback of PCR is that legally allowed ingredients which contain DNA, such as milk and milk products, will be detected as well (EFSA, 2007; Prado *et al.*, 2007; Yancy *et al.*, 2009; Cawthraw *et al.*, 2009; EFSA, 2011).

Near infrared detection

NIR spectroscopy is capable of detecting animal protein at and over a contamination level of 1%, which is higher than the technical requirement of 0.1% in EU. An advantage of NIR spectroscopy is its potential for the screening of large volumes of samples, thus improving the sampling strategy and reducing the sampling error, which is often an important factor in the total measurement error (Pérez-Marín *et al.*, 2006, 2008).

NIR microscopy analyses the NIR profile at the level of single pixels, which are pooled for all the pixels covering a single particle. This process is time consuming. A limit of detection of 0.1% PAPs in feed can be obtained on the analysis of the sediment fraction (Boix *et al.*, 2012). In all cases of NIR analysis, the specificity is the distinction between fish material and land animal material (Fumière *et al.*, 2009; Pavino *et al.*, 2010).

An advantage of NIR-techniques is that they are non-destructive. On the other hand, the main limitation is that a spectral library has to be constructed, consisting of a sufficient number of representative samples that capture the variability in the application area (Pérez-Marín *et al.*, 2009).

Immunoassays

Antibodies are available for detection of ruminant muscle fibre (troponin I). The current detection limits are close to 0.1%. Two methods, an ELISA assay and a lateral flow device, have been validated in an interlaboratory study. The combination of tissue and species specificity means that allowed ingredients are not detected, such as milk and blood products (van Raamsdonk *et al.*, 2012a; Bremer *et al.*, 2013; van Raamsdonk *et al.*, 2015).

Combination of methods

Immunoassays and PCR reactions can be achieved in situ, i.e. localised on a slide with animal proteins and examined by using a microscope. The proof of principle of in situ identification of ruminant muscle fibres with a labelled antibody was successful (van Raamsdonk *et al.*, 2011). DNA in ruminant bone fragments of processed animal proteins appeared to be amplified successfully in an in situ procedure (Lecrenier *et al.*, 2014). In both cases the background of the detected and identified particles was fully established. As an alternative to a localized identification, the target can be extracted from a slide by microdissection and used as basis for a PCR reaction (Axmann *et al.*, 2015).

Although bone particles had been identified on an individual basis by Near InfraRed Microscopy (NIRM), PCR on a whole particle basis after applying cleanup procedures revealed that ruminant milk and blood products were still detected (Fumière *et al.*, 2010).

Final remarks

- With respect to specific products, blood products and gelatin provide issues, since both products can originate from ruminant and non-ruminant sources, which are treated in a legally different way. Both PCR and Mass Spectrometry can be applied to identify and discriminate between these sources (Cai *et al.*, 2012; Flaudrops *et al.*, 2015).
- It is recommended to invest in methods which are both species specific and tissue specific. Raising antibodies is a costly procedure, but this will allow ELISA assays or lateral flow devices which can be applied in a routine and relatively cheap way.
- A global harmonisation of analytical methods is recommended.

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PHYSICAL HAZARDS

Materials in a broad sense that can induce physical hazards range from glass, metallic objects (e.g. nails and wires) and concrete from building materials, to former food products (FFPs) containing non-food materials (Papargyropoulou *et al.*, 2014; Anteneh and Ramswamy, 2015). Another major source of physical contamination of feed and food is the presence of micro- and nano-plastics as environmental pollution. These particles will enter the feed and food production chain (Bouwmeester *et al.*, 2015). The sizes may vary; particles smaller than 1 um are usually subject of the area of nanotechnology. In the current practice of food production, proper packaging of food is provided for assuring quality maintenance during transport and storage. Although there is a prohibition on the use of feed ingredients containing remnants of packaging materials in certain parts of the world, there is still an ambition in the framework of sustainability to use certain types of unpacked and processed FFPs as feed ingredients (Papargyropoulou *et al.*, 2014).

Current methods of analysis

Detection of microparticles, glass and metallic objects is typically based on visual examination (van Raamsdonk *et al.*, 2012; DeKiff *et al.*, 2014). A differentiation should be made for different particle sizes. Particles larger than 1 mm can be observed visually or by using a binocular, particles with a size between 1 mm and 1 um should be observed using a compound microscope, and below 1 um (nanoparticles) visible light is not eligible for examination. Quantification can be achieved for particles larger than 1 mm. The basis for examination is primarily the division of the sample material in several sieve fractions with different size ranges, complying to the principle that sample material with a homogeneous size distribution can be examined in a more reliable way (van Raamsdonk *et al.*, 2012; Choy & Drazen, 2013). In those cases where the matrix can be dissolved the physical contaminant can be extracted by using warm or hot water (Clauss *et al.*, 2011; Lauper *et al.*, 2013; RIKILT validated method for sugar syrup, unpublished). The separation of fractions according to their specific density can be applied additionally (Liebezeit and Dubaish, 2012; Cauwenberghe *et al.*, 2013). In experimental studies non-visual detection was achieved by adding a chemical marker, e.g. chromium (Hebel *et al.*, 2011). Identification of polymer types and additives in marine microplastic particles can be achieved using pyrolysis-GC/MS (Fries *et al.*, 2013).

Detection methods for nanoplastics are generally lacking (Bouwmeester *et al.*, 2015). Physico-chemical methods such as chromatography and mass spectroscopy have been applied for characterization of other nanoparticles (Peters *et al.*, 2011; Helsper *et al.*, 2013). Other techniques such as electron microscopy can be added to the physico-chemical techniques.

Final remarks

The detection of microparticles and of nanoparticles should follow different strategies. A harmonised set of detection and identification methods for microparticles is achievable based on discarding matrix material if possible (dilution, centrifugation), separation on physical density, and making sieve fractions. Most promising detection methods for nanoparticles might be based on dedicated physico-chemical methods.

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