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Chemical Residues in Animal Food Products: An Issue of Public Health

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1. Introduction

Human beings consume protein-rich foods to supply their nutritional requirements, mainly of animal origin, such origin lying in meat from different species (cattle, sheep, caprines, birds, pigs, fish and seafood/shellfish), milk and eggs. With the exception of some products derived from fishing, these foods are obtained from financial exploitations in which the animals' health must be guaranteed, thereby ensuring that food is harmless. In several countries the safety of such food has mainly been focused on avoiding the transmission of zoonotic diseases, less attention thus being paid to potentially present chemical residues, perhaps due to the course of the resulting disease. Whilst infectious processes are frequently of the acute type, toxicosis caused by contaminants in foods (more than acute) may be chronic, silent and often lacking a known aetiological agent.

The primary production of such food involves the animals' interaction with their setting from which they may become exposed to undesirable chemical substances which may generate residuality. The chemical substances to which animals may become exposed during their production cycle which have been identified to date could come from drugs and growth promoters aimed at treating diseases and improving production parameters, biologically-derived toxins (mycotoxins, phycotoxins, phytotoxins) and/or environmental contaminants linked to atmospheric pollution, from the soil and/or water. This chapter will be orientated towards dealing with residues from chemicals substances in foodstuffs of animal origin caused by drugs and growth promoters, as well as by toxins having a biological origin. It will also deal with general concepts such as toxic agent's target in an organism, the regulation of residues in food and the analytical methods used for detecting them. The contamination of food by chemical risks is a worldwide public health matter which may also hamper international trade.

2. The destination for toxic agents in an organism

Living beings continuously are being exposed to external substances, generically called xenobiotics, which can have adverse effects according to their chemical characteristics. Oral, dermal and inhalation routes represent the commonest means of exposure to these substances, the first being of interest as it deals with risks to human health due to the consumption of foodstuffs contaminated by potentially toxic substances. On the other hand, animals (representing a readily available source of food for humans) are exposed to

xenobiotics in multiple ways which could be present in their products. If one is dealing with veterinary drugs then the route of exposure could be oral (for example, coccidiostatics in poultry), dermal (e.g. external antiparasitic agents in ruminants), parenteral (e.g. antibiotic treatment in large animals) and even inhalation (if the animals are given anaesthesia before surgical procedures). Biologically-derived toxins mainly enter food-producing animals by the oral route (e.g. forage contaminated with mycotoxins or fish consuming toxic algae).

Xenobiotics in an organism go through a series of stages including absorption, distribution, metabolism and excretion, forming part of the pharmacokinetics or toxicokinetics according to the effects produced by a particular substance (pharmacological or toxicological). Xenobiotics enter a food-producing animals' organism and, according to its kinetics, reach the tissues which will become food for human beings (Figure 1). These concepts will be dealt with below, approaching them from the perspective of potential residuality which different substances can cause in an animal's organism.

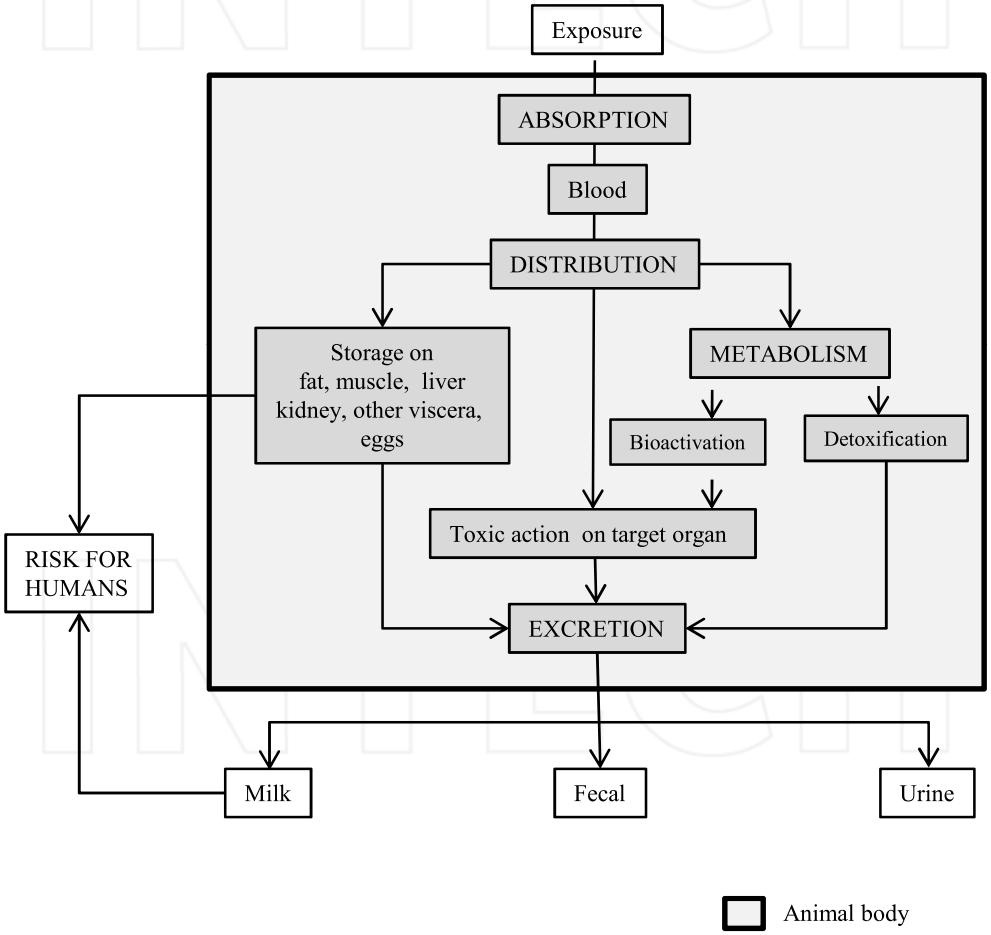

Fig. 1. Destination of toxicants in foodstuffs animal organism and risk for humans

2.1 Absorption

During absorption, the xenobiotics cross cell membranes and reach the systemic circulation. Many toxic agents enter with foodstuff and are absorbed by the same routes as the other substances present in them. The chemicals cross the cell membrane's lipid bilayer through two basic processes: diffusion (favouring their concentration gradients) and active transport (against their concentration gradients). Most liposoluble xenobiotics are transported by simple diffusion through the cell membranes. Organic acids and/or bases thus tend to become absorbed when they are in their most liposoluble form (non-ionised), which is determined by surrounding pH. Weak acids will become more easily absorbed in the stomach whilst this will happen to bases in the intestines. Hydrophilic substances having a small molecular weight become diffused through aqueous pores formed by proteins (facilitated diffusion). Active transport, against concentration gradients requiring an expenditure of energy, occurs through proteins present on the membrane mobilising a substance from one side to another (Lehman-McKeeman, 2008).

It should be born in mind that certain factors may sometimes alter xenobiotics' absorption; for example, the flora present in the gastrointestinal tract may transform them and leave them less available for being absorbed. This is why ruminants are resistant to some mycotoxins. Pre-systemic elimination of a toxic agent may occur with enterohepatic circulation, thereby minimising its potential adverse effect.

2.2 Distribution

Once it has been absorbed, a toxic agent becomes distributed throughout the whole body; during its initial phase this distribution is dominated by the blood flow. The penetration of toxic agent into the cells depends on passive diffusion or specialised transport; however, certain toxicants do not cross the membranes and become distributed via the blood flow. Some become accumulated in determined parts of the organism as a result of their binding to proteins or their high solubility in fat. When a toxicant becomes stored, then equilibrium is reached with the free fraction which is in the plasma. Thus, when the chemical becomes metabolised or is excreted, then substance is released from the storage site, thereby meaning that the xenobiotic half-life could become very long.

Albumin is the main plasmatic protein transporting xenobiotics. This protein may also be a toxicant reservoir since it impedes transport through the membranes due to its high molecular weight. The presence of toxic agents in the blood could be exploited for recognising exposure, whether in humans or animals.

Many organic compounds are very stable and lipophilic, becoming accumulated in the environment, becoming rapidly absorbed and concentrated in body fat. The toxicants become accumulated in fat because they are dissolved in it. A substance stored in fat is not toxic for the carrier, but there is rapid lipid mobilisation, for example poisoning may occur during long periods of fasting. Animal fat, a potential reserve of liposoluble toxicants, could be consumed by human beings.

The liver and the kidneys have a large capacity for proteins to bind a broad range of chemicals. These organs important function lying in the metabolism and elimination of xenobiotics makes them concentrate more toxic agents than all the rest combined. Thus consuming such viscera may represent a risk for the end consumer. There is a lower

presence of residues in animals' musculature (meat) compared to the viscera (kidneys, liver) and fat. Their accumulation at an injection site is feasible in cases where there has been exposure to the intramuscular drug route, this being important in animals which are to be consumed by humans.

The distribution of some chemicals in eggs, as well as reducing palatability, could represent a risk for the end consumer.

2.3 Metabolism

The object of xenobiotics' metabolism is to increase characteristics regarding an increase in substances' hydrosolubility so that they can be more easily excreted. This process occurs in two phases; hydrolysis, reduction and oxidation reactions are presented during phase 1, most of them being enzyme-mediated. Cytochrome P450 (CYP450) oxidation enzymes being of particular importance during this phase due to their catalytic versatility and the great number of xenobiotics constituted in their substrate. Conjugation reactions occur during phase 2, mainly with glucuronic acid, glutathione conjugates and sulphates; such reactions are enzymatically mediated by protein superfamilies called, respectively, uridine diphosphate glucuronosyltransferase, glutathione S-transferases and sulphotransferases. In spite of the initial purpose of xenobiotics' metabolism (or biotransformation) being detoxification, substances can occasionally acquire their true toxic power on being biotransformed; such reaction is called bioactivation or metabolic activation. Aflatoxins and pyrrolizidine alkaloids are bioactivated substances of interest regarding the residuality which they represent in food of animal origin.

2.4 Excretion

Toxicants are eliminated from the body by various routes, the kidneys being the most important organ for excreting xenobiotics since it is the main elimination route. The biliary route involving the faeces is the other elimination route for toxic substances which have been consumed.

Milk is an important elimination route due to the risk of contamination which it represents; this liquid is a lipid emulsion in an aqueous protein solution and may thus contain whatever toxicant which is in solution in an animal's body water. Simple chemicals arrive at the mammary glands by diffusion in their free form, bound to proteins or dissolved in lipids. The percentage of the total amount of compounds eliminated in milk is very low because the other elimination routes are more efficient. However, the main problem lies in chronic exposure and/or liposoluble compounds (Panter & James, 1990).

The concept of withdrawal time has been established to avoid the accumulation of drug residues in animals; it is defined as being the time required after a drug has been administered to an animal to ensure that drug residues in marketable products (meat, eggs, viscera or other edible products) are below a determined maximum residue limit (MRL).

3. Regulating and evaluating risk

There can never be an absolute guarantee that our food is safe; it is simply impossible to test every contaminant. Every country has an agency which oversees food safety; this is defined

as being the, "reasonable certainty of no harm," and the aforementioned agencies regulate which additives are allowed in food and what levels of unavoidable contaminants are acceptable. The US Department of Agriculture's (USDA) Food Safety Inspection Service (FSIS) is responsible for the safety of meat, poultry, and egg products in the USA (Lodovico et al., 2008). The European Food Safety Authority (EFSA) is the keystone of the European Union's (EU) risk assessment regarding food and animal feed safety. The Codex Alimentarius Commission (created by the FAO and WHO) develops food standards, guidelines and related texts such as codes of practice under the Joint FAO/WHO Food Standards Programme (JECFA). The main purposes of this programme are protecting consumers' health, ensuring fair trade practices in the food trade and promoting the coordination of all food standards' work undertaken by international governmental and non-governmental organisations.

Health authorities recommend maximum acceptable or tolerable levels for chemicals which are neither genotoxic nor carcinogenic, such as acceptable daily intake (ADI), reference dose (RfD), especially for pesticides, tolerable daily intake (TDI) and provisional tolerable weekly intake (PTWI) for contaminants which may accumulate in the body. The responsible agencies conduct risk assessment to determine such levels; this consists of hazard identification and characterisation, exposure assessment and subsequent risk characterisation.

Hazards are identified and characterised from human epidemiological observations and animal-based toxicity testing supported by *in vitro* mechanistic studies which can make extrapolation from animals to humans become more realistic. Structure–activity relationships-based indications and the increased use of novel molecular biology techniques are also very valuable.

Dose–response information is essential for quantifying an adverse health effect. This may be graphically presented as being the relationship between the increase of a dose and the increase of a pertinent biological response. Such dose–response curve is essential for identifying a non-active dose taken as being the no observed adverse effect level (NOAEL), the highest dose of a substance which causes no detectable adverse alteration in line with defined treatment conditions. Interspecies differences should be taken into account as well as the fact that humans may exhibit substantial differences in their sensitivity to certain toxins due to differences regarding metabolic pathways and other factors. Uncertainty factors are thus applied when extrapolating from the toxicity observed in laboratory animals to health risks in humans, this usually being a factor of 10 for interspecies difference and a factor of up to 10 for human variability (depending on the extent and quality of available human data).

The resulting value (equation 1) provides an estimate of the amount of a substance in food, expressed on a body weight basis, that can be ingested daily over a lifetime without appreciable risk (standard human= 60 kg). The ADI is then used for determining the maximum allowable levels of a particular chemical in a specific food, depending on the extent to which this food contributes towards the overall intake of such chemical (Lodovico et al., 2008). These are called maximum limits for some chemicals and maximum residue limits (MRLs) for substances such as veterinary drugs and hormone residues.

$$ADI = \frac{NOAEL}{UF}$$

ADI : Acceptable Daily Intake
NOAEL : Not Observed Adverse Effect Level
UF : Uncertainty Factor (10, 100, n)

Equation 1. Acceptable daily intake calculation

The regulatory approach (not strictly scientific) for genotoxic and carcinogenic compounds is based on the assumption that there is no threshold dose (it is assumed that one genotoxic molecule is sufficient to hit a single DNA base, thereby inducing damage). The aim in this case is to keep the exposure level as low as technologically achievable (Lodovico et al., 2008).

4. Analytical methodologies

Analytical data quality is a key factor in the success of a control programme dealing with residues in foodstuffs. The analytical results of methods regarding official standards offer the necessary information for developing and managing programmes responding to a population's public health needs. It is very important that sanitary authorities have readily available practical analysis methods which will reliably detect and quantify (without ambiguity) a drug's residues which could be present in meat, milk, or eggs at a suitable concentration level. Unfortunately, methods having these attributes are not available for all residues, partly due to the large amount of possible substances which may be found in animals' food chains.

Chemical residues in food of animal origin, such as meat, milk or eggs, are frequently present in very low concentrations or trace levels, thereby representing an important challenge for a chemical analyst, given that the analytical methods developed must be highly sensitive and selective.

The prior treatment which a sample has received is very important for ensuring that methods reach desired detection levels as well as an acceptable level of exactitude and precision, thereby enabling the factors responsible for analyte loss to be controlled during such procedure (The Spanish Industrial Pharmaceutics Association – Asociación Española de Farmacéuticos de la Industria [AEFI], 2001); this would include the presence of functional reactive groups which can interfere with such determination (LoBrutto & Patel, 2007).

Analytical methods will always have to be tested/proved on material from each animal species since differences in composition (fat, specifics proteins, eg: myoglobin) can influence both analyte extraction and separation. Another important consideration concerns the treatment which biotransformation enzyme-rich tissues such as the liver should receive as this may induce post-mortem metabolism thereby altering real results. Special management musts also be used for determining analytes in eggs because eggs consist of two distinct compartments (the white and the yolk) whose chemical composition is different, as well as depending on the components of chicken's diet.

The scientific community focuses on developing reliable, economic and rapid methods (and whenever possible automated) which could be applied to evaluating the safety of foodstuffs bearing in mind the broad range of existing chemicals and matrices (Botsoglou & Fletouris, 2000). A unified procedure would eliminate the need for using separate methods for detecting multiple residues in the same product; however, such methods are rarely found in real life. There is an immense variety of methods for identifying, confirming and quantifying analytes which could be used individually or coupled to each other in a suitable way. These methods can be grouped into bioassays, microbiology assays, immunochemical assays and physical-chemical assays.

4.1 Bioassays

Biological methods for determining toxic residues in foodstuffs can be used both *in vivo* and *in vitro* (FAO, 2004) and have been particularly developed for detecting and quantifying the phycotoxins present in shellfish. The mouse bioassay is the most used one and is even accepted by regulating entities.

A toxin extract is intraperitoneally injected into mice having around 20 g body weight in the mouse bioassay and their survival is monitored from 24 to 48 hours. One mouse unit (MU) is defined as being the minimum quantity of toxin needed to kill a mouse within 24 hours. Sample toxicity (MU/g whole tissue) is determined from the smallest dose at which two mice or more in a group of three die within 24 hours. The regulatory level is set at 0.05 MU/g whole tissues in many countries; this assay's major disadvantages are therefore a lack of specificity (no differentiation between various toxin components), subjectivity regarding the animals' time of death as well as maintaining and killing laboratory animals. This assay may also give false positives because of interference which can be very toxic for mice. The EU has issued directions on how to perform this assay in an attempt to standardise the mouse bioassay methodology.

Other bioassays which are also used would include the suckling mouse assay for detecting marine toxins which determin the weight of the intestine regarding body weight, the rat bioassay which is based on inducing diarrhoea in rats, the *Daphnia magna* assay which is used for detecting okadaic acid, the intestinal loop assay which determines the accumulation of fluids in rabbit intestine and mice and cytotoxicity assays which are based on observing morphological changes in cells.

4.2 Microbiological assays

The microbiological methods used for detecting antimicrobial residues in foodstuffs are based on inhibiting microbial growth, microbial receptor activity and enzymatic reactions and could be applied to all types of matrices, usually milk, meat, eggs and honey. Microbial inhibition assays involve culturing a microorganism from a standard strain, usually *Bacillus stearothermophilus, Bacillus subtilis, Bacillus cereus, Micrococcus luteus, Escherichia coli, Bacillus megatherium, Sarcina lutea* and/or *Streptococcus thermophilus* (AEFI, 2001).

4.3 Immunochemical assays

Immunochemical methods represent an important tool for determining drug residues, given their high specificity, they lead to analytes being determined in samples having had very

reduced prior cleaning treatment. These assays are based on the reaction of an antigen binding to a specific primary antibody or for each antigen, analogously to an enzyme-substrate reaction. The most common immunochemical methods would include the enzyme-linked immunosorbent assay (ELISA), direct and indirect competitive enzyme-linked immunosorbent assays, immunoaffinity chromatography (IAC), radioimmunoassay (RIA), the enzyme-monitored immunotest (EMIT), the fluorescent immunoassay (FIA) and the chemiluminescence immunoassay.

4.4 Physical-chemical assays

Physical-chemical methods are mainly used for isolating, separating, quantifying and confirming the presence of dangerous residues in samples; this requires that the sensitivity of a particular selection method and the determinative or confirmation method are similar. Numerous procedures based on the analytes'different physicochemical properties have been developed for achieving this objective. Even though a drug's chemical structure greatly determines the most suitable method for its determination, different methods are usually available for the same analyte due to the large amount of possibilities and by coupling different methods to obtain optimum analyte separation and detection.

Separation methods are based on the principles of chromatography and are generally coupled to high sensitivity and selectivity detection techniques leading to quantifying an analyte with a high level of precision and exactitude and also its unequivocal identification at very low concentration levels. The chromatographic methods used for determining analytes in complex matrices would be gas chromatography (GC), high performance liquid chromatography (HPLC), ionic chromatography (IC), size exclusion chromatography (SEC), supercritical fluid chromatography (SFC), affinity chromatography (AC).

Spectrometric methods are also used either alone or coupled to chromatographic or immunochemical methods such as ultraviolet-visible absorption spectrometry, absorption spectrometry in the near and middle infrared sections, fluorescence and chemiluminescence spectrometry, X-ray fluorescence spectrometry, atomic absorption spectrometry, atomic emission spectrometry (AES), inductively-coupled plasma atomic emission spectrometry (ICP-AES), nuclear magnetic resonance (NMR), mass spectrometry (MS) and mass spectrometry in tandem (MS/MS) (Mastovska, 2011).

Other separations methods are used in determined analysis such as capillary electrophoresis (CE), electro capillary chromatography (ECC) and polarimetry (Rouessac & Rouessac, 2003).

5. Veterinary drugs and growth promoters in food of animal origin

Currently, rearing animals aimed at feeding the human population mainly depends on using pharmacologically-active compounds. Using drugs in the animals is fundamental for animal health and wellbeing and for the economy of agribusiness. The reported benefits are mainly derived from keeping animals in good health, thereby reducing the possibility of a disease becoming transmitted from animals to humans. However, residues from drugs used in producing food of animal origin could increase the risk of disease in the people who consume products from treated animals.

In principle, all pharmaceutical preparations administered to animals producing foodstuffs can give rise to residues in edible tissue, milk or eggs. In addition to drug dose, residue

levels depend on withdrawal time. In spite of most drugs representing a relatively low risk for the general public, when used responsibly and in line with instructions approved by the laboratories making veterinary drugs, adverse reactions have been frequently reported for some compounds; these would include antibacterial, antihelminthic, anticoccidial and antiprotozoal drugs and growth promoters.

5.1 Antibacterial drugs

Residues from antibacterial drugs in food products of animal origin can represent a danger for consumers. The poisonous effects are not very probable since the residues are present in very low concentrations. Some substances must receive particular attention due allergic reactions. The main hazardous effect is likely to be the development of resistant bacterial strains following sub-therapeutic doses of antimicrobials being ingested; such resistance could be transferred to other bacteria. This could include resistance being transferred from non-pathogenic organisms to pathogenic ones which would then no longer respond to standard drug treatment (the Institute of Food Technologists [IFT], 2006). However, the generally used antibacterial drugs are presented in Table 1.

Differences in substitutions in the basic ring structures between the various aminoglycosides account for the relatively minor differences in antimicrobial spectra and resistance and toxicity patterns. Aminoglycosides given in therapeutic dosages mainly cause ototoxicosis, but may also cause nephrotoxicosis, allergy and neuromuscular disturbances.

Chloramphenicol (an antibacterial belonging to the amphenicol group) has been used in treatment and prophylactically in food-producing animals for several years now (i.e. poultry, calves, pigs, sheep and fish). Chloramphenicol's most serious toxic effect is bone marrow depression which is generally dose-related and reversible but can sometimes be fatal in patients who are probably genetically predisposed. A toxic syndrome has been reported in newborn infants receiving large doses of chloramphenicol which is characterised by vomiting, hypothermia, cyanosis and circulatory collapse followed by death; this syndrome rarely occurs in adults. Chloramphenicol may also cause neuritis, encephalopathy with dementia and ototoxicity; its use is restricted in many countries, while it is totally banned for use in food-producing animals within the European Union and the USA. Chloramphenicol and its metabolites could be genotoxic (Lozano & Arias, 2008).

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Antibacterials	Aminoglicosides	Streptomycin, kanamycin, amikacin, neomycin, apramycin
	Amphenicols	Chloramphenicol, thiamphenicol, florfenicol
	Beta-lactams	Penicilins, cephalosporins
	Macrolides	Erythromycin, spiramycin, kitasamycin, josamycin, desmycosin, mirosamycin, tilmicosin, leucomycin, tylosin
	Nitrofurans	Furazolidone, nitrofurazone, furaltadone, nitrofurantoin
	Quinolones	Ciprofloxacin, danofloxacin, difloxacin, enrofloxacin, flumequine, marbofloxacin, norfloxacin, ofloxacin
	Sulphonamides	Sulfadiazine, sulfadimethoxine, sulfamethazine, sulfadoxine, sulfaethoxypyridazine, sulfaguanidine, sulfamerazine, sulfamethoxazole, sulfapyridine, sulfamethoxydiazine, sulfamethoxypyridazine, sulfamonomethoxine, sulfathiazole, sulfaquinoxaline
	Tetracyclines	Chlortetracycline, oxytetracycline, demeclocycline, doxycycline, methacycline, minocycline
Antihelmintics	Benzamidazoles	Thiabendazol, flubendazol, fenbendazol, mebendazol, albendazol, oxfendazol, febantel
	Imidazotiazoles	Levamisole
	Organophosphates	Haxolon, coumaphos, dichlorvos
	Tetrahydropyrimidines	Morantel, pyrantel
	Salicylanilides	Closantel, niclosamide, oxyclozanide, rafoxanide
	Sustituted phenols	Dichlorophen, hexachlorophen
	Macrocycliclactones	Abamectin, ivermectin, moxidectin
	Piperazinederivates	Piperazine, diethylcarbamazine
Antiprotozoals	Benzamides	Aklomide, nitromide, dinitolmide
	Carbanilides	Nicarbazin, imidocarb
	Nitroimidazoles	Ronidazole, dimetridazole, metronidazole, ipronidazole
	Polyether ionophore	Monensin, narasin, lasalocid, salinomycin, maduramicin
	Quinolonederivates	Buquinolate, decoquinate, methylbenzoquate
	Triazines	Clazuril, diclazuril, toltrazuril
Growth promoters	Antibiotics	Monensin, salinomycin, bambermycin, avilamycin
	Anabolic hormones	Estradiol-17, progesterone, testosterone
	Synthetic steroidal	Boldenone, chlormadinone acetate, ethylenestrol, fluoxymesterone, medroxyprogesterone acetate, megestrol acetate, methandienone, methylboldenone, methyltestosterone, drostanolone, norethandrolone, norgestomet, norgestrel, nortestosterone oxymetholone
	Organic arsenicals	Arsanilic acid
	Peptide antibiotics	Avoparcin, bacitracin, efrotomycin, enramycin, thiopeptin
	Quinoxaline-1,4-dioxides	Carbadox, olaquindox
	Beta-adrenergic agonists	Bambuterol, bromobuterol, carbuterol, cimaterol, clenbuterol, dobutamine, fenoterol, isoproterenol, mabuterol, mapenterol, metaproterenol, pirbuterol, ractopamine, reproterol, rimiterol, ritodrine, salbutamol

Table 1. Drugs administrated in treatment and prophylactically in food-producing animals

Penicillins have low toxicity; hypersensitivity reactions, especially skin rashes, are by far their most common adverse effects. Gastrointestinal disturbances including diarrhoea, nausea and vomiting may also sometimes appear. No teratogenic effects have been reported. Some studies have indicated that sensitive people have experienced allergic reactions, such as genera pruritis (itching), difficulty in swallowing and talking, dyspnoea,

dermatitis caused by contact and urticaria (hives) caused by consuming residues present in meat and/or milk (Medina et al., 2008). The allergic reactions caused by penicillin and its derivatives have been considered by the JECFA committee as being determinant factors for evaluating and establishing safe residue levels in foodstuffs. The adverse effects associated with cephalosporins are similar to those described for penicillins.

Lincomycine-macrolide is used for the initial treatment of mild to moderate staphylococcal infections in calves, sheep, goats and pigs and it is also added in feed for growth-promoting purposes. Lincomycine is reported to cause gastrointestinal disturbances including diarrhoea, vomiting and nausea which that may prove fatal. Other adverse effects include skin rashes, urticaria, polyarthritis, hepatic damage and haematological disturbances (WHO, 1989).

All nitrofurans have been widely used in the prophylactic and therapeutic treatment of infections caused by bacteria and protozoa in pigs, cattle, poultry, rabbits and fish. The use of nitrofurans in food-producing animals has been controversial because residues from these drugs may be mutagenic and tumorigenic. Toxicological studies have shown that nitrofurazone is a carcinogenic but not genotoxic agent, whereas furazolidone has exhibited both carcinogenic and genotoxic properties (WHO, 1993). The metabolites from nitrofurans can remain stored for weeks or m animal proteins, including eggs from farmyard birds, species in which this compound has been used as an anticoccidial. The systemic use of nitrofurans in food producing animals has thus been prohibited in the USA and Europe, (EMEA, 1997).

Quinolones are synthetic antibiotics which are very effective in combating various diseases in animal husbandry and aquaculture. The most frequent adverse affects of quinolone antibiotics most frequently occurring adverse affects are gastrointestinal disturbances including nausea, vomiting, diarrhoea, headache, visual disturbances and insomnia. Rashes, pruritus and epidermal necrolysis have sometimes also occurred (Jimenez et al., 2011).

The residuality of sulphonamides used in treating coccidial and bacterial infections and also as growth-promoting agents may cause hypersensitivity reactions, mainly cutaneous rash; however, no anaphylactic manifestations caused by this type of residues is known.

There is sufficient evidence indicating that ingesting the antibiotics in sub-therapeutic doses makes a significant contribution towards the appearance of resistant microorganisms in animals which can become transmitted to humans, thereby provoking difficult to treat infections. Some sample studies have found antibiotic-resistant coliform microorganisms in raw and cooked meat. Likewise, antibiotics consumed by human beings from residues present in food of animal origin lead to an alteration of intestinal flora and consequently a reduction of bacteria competing with pathogenous microorganisms, thereby increasing the risk of disease.

Tetracyclines can generate bacterial resistance; oxytetracycline particularly induces antibiotic resistance in coliform microorganisms present in the human intestine. Recognition of this effect has been used by the JECFA committee as the point of reference for defining acceptable consumption levels for different antibiotics.

The problem of resistance is not the only motive for the medical community's preoccupation. Farmers and veterinarians are worried because bacterial resistance in farm

animals is interfering with drug efficacy thereby leading to the use of greater concentrations than those initially established as being therapeutic. However, in spite of antibiotics being the type of veterinary drugs most used in the agribusiness industry, there are few options to choose from due to the limited offer of drugs which have been approved for use in animals producing foodstuffs compared to those regarding therapeutic use in humans.

5.2 Antihelminthic, anticoccidial and antiprotozoal drugs

Parasitic diseases constitute an ever present threat in rearing birds and livestock, but they may be controlled by adding low levels of drugs to daily rations. The drugs generally against internal parasites affecting animals collectively called helminths are shown in Table 1. Such drugs are used at levels which do not allow resistant strains to develop and also become rapidly metabolised in an animal's organism so that the residues in edible tissues are minimal.

Benzimidazoles, like thiabendazole, are used in sheep, cattle, horses, pigs and poultry. They become rapidly eliminated from the organism due to their high solubility; however, some studies has shown that these compounds are teratogenic and nephrotoxic in mice and ewes (Danaher et al., 2007). Mebendazole metabolits (hydroxylmebendazole and amino-mebendazole) belonging to the benzimidazole group and which are widely used as an antinematode in horses, sheep, pigs and poultry, have also been shown to have teratogenic effects (Buchmann et al., 1992).

Levamisol is the most well-known drug from the imidazothiazole group, which has a broad spectrum of activity against nematodes; however, it has been found that it induces idiosyncratic organulocytosis in some individuals. Levamisol's toxic effects have caused preoccupation in the regulatory bodies and, given that these effects the original compound than in its metabolites, this is the analyte of interest in tissue samples.

Organophosphates represent one of the alternatives for treating benzimidazole-resistant nematodes; haloxon (being one of them) is the safest and has been approved by the US Food and Drug Administration (FDA) for use in sheep, cattle and goats. By contrast, dichlorvos has an acceptable antihelminthic spectrum in cattle and sheep, but it does not have FDA approval for use in ruminants due to its suspected carcinogenic effects and narrow safety margin (Botsoglou & Fletouris, 2000).

Ivermectin, a macrocyclic lactone, is exceptionally effective in very low dosages against nematodes and arthropod parasites in cattle and has been widely used for treating endo- and ecto-parasites in cattle, sheep, goats and pigs; however, ivermectin has had a teratogenic effect in rats, rabbits and mice (Moreno et al., 2008).

Anticoccidial and antiprotozoal drugs are generally used in the poultry industry against protozoan infections caused by pathogenic species of *Eimeria.* Some compounds used as antibacterial drugs are also used as coccidiostats, including sulphaquinoxaline, sulphadimethoxine, sulphamethoxypyridazine, sulphachlorpyrazine, sulphamethazine, sulphaguanidine, furazolidone, nitrofurazone, tetracycline and chlortetracycline. Table 1 shows compounds whose primary function and use are related to antiprotozoal drugs.

A number of nitroimidazoles have already been banned within the European Union, even for therapeutic purposes, since they are mutagens and suspected carcinogens. The use of ronidazole has been banned by Council Regulation 3426/93/EEC (Official Journal of the European Communities, 1993) whereas dimetridazole use is banned by Council Regulation 1798/95/EEC (Official Journal of the European Communities, 1995). Their antibacterial and mutagenic activity is closely related to the reduction of the 5-nitro group, which is common to all nitroimidazole drugs. Metronidazole is used for treating bovine trichomoniasis by topical application or intravenous injection but it is a genotoxic carcinogen in animals.

Polyether antibiotics are produced by various actinomyces, mostly *Streptomyces* species, and constitute the agents most widely used by the poultry industry over the last two decades. They provide excellent disease control and are refractory for the development of resistance. They have a low therapeutic index but may be very toxic in certain species; salinomycin and narasin can be fatally toxic in turkeys, for example (Weissinger, 1994).

The problem of residues from antihelminthic, anticoccidial and antiprotozoal drugs may be easily controlled by imposing obligatory withdrawal times, generally 7-10 days, but this is unfortunately not always respected. On the other hand, given the large number of drugs which may be easily obtained on the market, many producers change one compound for another to avoid resistance becoming developed to drugs; however, this increases the degree of exposure to thema the misms, which may lead to yet another problem if it is taken into account that these drugs are also used as growth promoters.

5.3 Growth promoters

Growth promoters are substances which produce improvements in growth rate when added to animal feed in sub-therapeutic dosages over an extended period of time. Table 1 shows the compounds most commonly used for this purpose. The anabolic hormonal-type growth promoters can be classified according to their chemical structure or origin into endogenous sex steroids, steroidal compounds, not naturally occurring non-steroidal compounds and polypeptide hormones.

Anabolic hormones (estradiol-17 and progesterone - two female sex hormones, and testosterone - one male sex hormone) are used for increasing body mass in livestock rearing. Synthetic steroidal compounds have only been approved for therapy regarding reproductive behaviour and disorders in non-food-producing animals; however, they are used illegally around the world. Boldenone, chlormadinone acetate, ethylenestrol, fluoxymesterone, medroxyprogesterone acetate, megestrol acetate, methandienone, methylboldenone, methylthisterone, drostanolone, norethandrolone, norgestomet, norgestrel, norethisterone (nandrolone), norethisterone decanoate, oxymetholone, and stanozolol would be examples of synthetic steroidal compounds which have only been approved for therapy regarding reproductive behaviour and disorders in non-food-producing animals.

Zeranol and stilbene estrogens, including diethylstilbestrol, hexestrol and dienestrol, are the major non-steroidal not naturally occurring compounds included in the class of anabolic drugs and somatropin is the most common polypeptide compound affecting growth. Diethylstilbestrol, hexestrol and dienestrol are all stilbene estrogens which are currently banned worldwide for use in food-producing animals. They are genotoxic, not easily

metabolised compounds, which are considered capable of irreversibly initiating the carcinogenic process even in small residue concentrations. Diethylstilbestrol and hexestrol have been legally permitted for use as anabolics for quite some time in many countries, whilst the use of dienestrol, which is a diethylstilbestrol metabolite, has been restricted to illegal practice (Dickson, 2003).

Using these compounds, either natural or synthetic, as growth promoters in meat-producing animals has not been allowed in the European Union since 1988, due to potential adverse effects to human health, unlike in the United States where some anabolic hormonal-type growth promoters are permitted.

In vivo studies have demonstrated DNA strand breaks and oxidative damage being triggered by desencadenados por the 17-β estradiol, thereby leading to this hormone being considered as triggering a genotoxic effect (for example, the proliferation of carcinogenic mammary cells); however, the dosage at which these alterations occur is greater than that at which endocrine effects are produced in animals (Mikus et al., 2001).

Testosterone's adverse effects are due to its hormonal activity, particularly in the prostate gland. Testosterone is also considered to be potentially embryotoxic and its consumption in therapeutic doses has led to the induction of hepatic cystitis (Durlinger et al., 2002).

Following the ban of stilbene and other hormonal-type growth promoters, interest has focused on alternative compounds for promoting live weight gain in food-producing animals. The beta-adrenergic agonists constitute such group of compounds, clembuterol being the main one. It has been reported that consuming calf liver in Spain and France containing clenbuterol residues has induced muscular tremors, tachycardia, muscular pain, nervousness, headache, vertigo, nausea, vomiting and fever. It has also been used as an anabolizant steroid; clenbuterol is used as a tocolytic in cows, thereby supposing an additional risk.

A controversy has arisen around these events regarding whether to accept or prohibit using clenbuterol in animal production. This drug increases channel performance, it is not potentially oncogenic or mutagenic and is only embryotoxic in large doses whilst its adverse effects on consumers becomes evident when recommended withdrawal times are not respected and when excessive doses are used, whether through inadequate management or aimed at increasing animals' weight gain even more (Brambilla et al., 2007).

The foregoing has led to clenbuterol being a highly controlled drug today in many countries which have developed programmes and mechanisms for monitoring it and its follow-up. However, in spite of these controls and warning signs, unfortunate events involving adverse reactions continue to be presented, as happened in November 2005 in Jalisco, México, when about 225 people experienced trembling, headaches and discomfort after having consumed beef containing residues of this type (Gojmerac, 2002).

Arsanylic acid, peptide antibiotics and quinoxaline-1,4-dioxides are non-steroidal compounds used as growth promoters in different animal species. Arsanylic acid and its sodium salt are most commonly used, particularly in pigs. They are also efficacious in the egg-producing industry and were previously approved for use in egg-laying hens. However, their use in animals is generally rather limited and the risk–benefit ratio is questionable because these drugs can produce toxicosis, known as peripheral nerve demyelination.

In spite of the aforementioned effects, the Codex Alimentarius considers it unnecessary to establish an LMR for anabolic hormones as it is improbable that residues arising from the correct used of these substances as growth stimulators represent a danger for human health. It has also been demonstrated that the endogenous concentration of these hormones is greater when they are administered exogenously. Another reason negating the potential risk of this type of substance is the availability of metabolic routes which become rapidly degraded, meaning that the residues which the meat of treated animals may contain do not affect a consumer's endocrine system. However, dispositions in Europe regarding these substances are stricter and do not allow any residual level of anabolizant drugs in meats.

Peptide antibiotics are compounds usually containing D-amino acids. They are usually added to animal feeds in low concentrations and produce residues in tissues at very low or undetectable levels. Unfortunately, most peptide antibiotics' metabolic pathways have not yet been elucidated. These antibiotics are regulated under separate legislation within the European Union (Brogden et al., 2003).

Quinoxaline-1,4-dioxides and their possible residues in edible animal products have caused much debate regarding their mutagenic and carcinogenic potency. Carbadox was initially the main drug in use, but suspicion as to its safety arose because this compound exhibited both genotoxic and mutagenic activity. Olaquindox is also a strongly mutagenic agent but seemingly devoid of carcinogenic activity.

6. Toxins in food of animal origin

Toxins have a biological origin, mycotoxins, phycotoxins and phytotoxins having attracted most attention due to their potential residuality in foodstuffs, including animal subproducts.

6.1 Mycotoxins

Mycotoxins are secondary metabolites from fungi, mainly from the species *Aspergillus*, *Fusarium* and *Penicillium*, aflatoxins, ochratoxins, zearalenone, trichothecenes and fumonisins having been the most studied to date. The foodstuffs fundamentally contaminated by these toxins are grains and cereals constituting the main source of contamination for human beings. However, farm animals consuming contaminated foodstuffs may generate residues in meat, viscera, milk and eggs. Residuality is determined by contamination by high concentrations in foodstuffs ingested by animals, this being very uncommon, and also by theway in which the xenobiotic becomes metabolised in the organism. Mycotoxins do not become totally destroyed during cooking or industrialisation of foodstuffs due to their heat-stability.

The types of mycotoxicosis (disease resulting from consuming mycotoxins) in human beings are mainly chronic. These would include Balkan endemic neuropathy in Russia caused by the consumption of ochratoxin A which generates nephrotoxicosis, alimentary toxic aleukia in the former Soviet Union associated with dermatitis, vomiting and hematopoietic alterations caused by trichothecenes (diacetoxiscirpenol and T-2 toxin), possible endocrinal alterations related to reduced masculine fertility caused by consuming zearalenone (such toxin acting as an xenoestrogen), hepatic cancer caused by aflatoxin B1 and possible esophagic and renal cancer caused by fumonisin and ochratoxin A, respectively. The IARC

has classified aflatoxin B1 within group 1 (proven carcinogenic effect on humans) and fumonisin B1 and ochratoxin A within group 2B (possibly carcinogenic to humans) (IARC, 1993). The evidence from *in vitro* studies has shown that zearalenone is a probably implicated in cancer of the reproductive system (Khosrokhavar et al., 2009).

Aflatoxins and ochratoxin A are the main mycotoxins which can generate residuality and attention concerning them as being animal subproduct contaminants has mainly been focused on their presence in milk; however, it has been demonstrated that they can also generate residuality in meat and eggs.

6.1.1 Aflatoxins

Aflatoxins (AF) B1 B2 G1 and G2 are produced by fungi from the genera *Aspergillus*. AFB1 may be bioactivated through CYP450 enzymes to become an epoxide which is able to form adducts with DNA, meaning that it has been considered that AFB1 undergoes bioactivation in the organism. AFB1 may also become hydroxylised to AFM1 and be excreted in milk. It has been estimated that 1% to 6% of AFB1 ingested by a milk-producing cow could be excreted as AFM1 in milk, depending on bovine productivity. AFM1, like AFB1, may form an epoxide and alter DNA sequences. IARC is considered to be an AFM1 in group 2B (IARC, 1993). MRL regulated in different countries ranges from 0.05 to 0.5 μg/L; MRL has also been established for AF consumed by ruminants (FAO, 2003). Experimental studies have shown that when animals consume foodstuffs contaminated by high levels of AF, that it is difficult to find naturally, the liver and kidneys are the organs where most toxins become accumulated, and their presence in muscle is scarce (Bailly & Guerre, 2009). These types of studies have also demonstrated the presence of AF in eggs from different avian species.

6.1.2 Ochratoxin A

Ochratoxin A (OA) is produced by fungi from the genera *Aspergillus* and *Pencillium*, the former being from tropical regions and the latter from temperate regions. OA may thus be widely distributed throughout the world. OA may become biotransformed through hydrolysis reactions in which metabolites become less toxic by the opening of the lactone ring which occurs during bioactivation. Detoxification may occur in ruminants through digestive flora action before absorption, thereby limiting the possibility that OA might be found in milk and/or beef (Bailly & Guerre, 2009). However, a recent study evaluating the presence of OA in cows' milk formulas for infants found contamination in 72% of the samples analysed, levels around 690 ng/L being found (Meucci et al., 2010). It has been shown that OA may become accumulated in pigs' kidneys. In countries such as Denmark, OA levels in these organs are regulated since porcine ochratoxicosis is common.

6.1.3 Fusariotoxins

The fusariotoxins are mycotoxins which are produced by fungi from the genera *Fusarium*, zearalenone (ZEA), the fumonisins (FUM) and the trichothecenes (TCT) being the most important for public health.

ZEA is frequently implicated in reproductive disorders in animals and occasionally in hyperestrogenism syndromes in humans. ZEA becomes biotransformed in the intestine by the mucosa or bacterial flora and involves the formation of α- and β-zearalenol and α- and β-zearalanol. Alpha-zearalanol and β-zearalenol have greater estrogenic power than ZEA since they bind with greater force to their corresponding receptors (Zinedine et al., 2007). Alpha-zearalanol has been employed as growth promoter in cattle. Studies orientated towards determining residuality through experimentation have suggested that residues are not present in meat or eggs, even at high doses. However, a recent study has show that the presence of α-zearalenol in meat-based foodstuffs for infants reached levels of 30.5 μg/kg; the same study demonstrated the presence of mycoestrogens (ZEA, α-zearalenole and β-zearalenole) in infants' cow milk formulas (Meucci et al., 2011).

FUM properties suggest that their presence in animal meat does not represent an important source of contamination, since they are poorly absorbed. FUM produce liquefaction of the brain in horse and pulmonary oedema in pigs; ruminants and birds are more resistant. They have been correlated with oesophageal cancer in humans in some parts of the world and it has also been presumed that they may cause neural tube alterations. Their presence has been demonstrated and in the liver and kidneys of turkeys fed with the maximum levels permitted in Europe (Tardieu et al., 2008).

The main TCT of interest in producing animals are T-2 toxin, HT-2 toxin, diacetoxiscirpenol and deoxinivalenol. The TCT do not usually represent a risk of contamination in food of animal origin due to their rapid metabolism (Bailly & Guerre, 2009).

6.2 Phycotoxins

Around 75 species of marine micro-algae, belonging to the dinoflagellate group, produce secondary metabolites which represent potent toxins, generically called phycotoxins. These organisms form part of the marine plankton and therefore the aquatic food chain leading to filtrator mollusks, gastropods, crustaceans and fish which can accumulate toxins being consumed (FAO, 2004).

The microalgae population may increase suddenly and generate an algal bloom which has increased in frequency, intensity and geographical distribution during recent years. Amongst the explanations put forward to explain for this phenomena has been the increased use of coastal waters for aquaculture, eutrophication caused by domestic, industrial and agricultural residues, the mobility of trace metals and humic substances due to deforestation and/or acid rain and changes in climatic conditions (Erdner et al., 2008). Reports of phycotoxins poisoning have increased during the last few years, perhaps due to the scientific community's greater knowledge and interest in the matter or due to the increase in algal bloom. Such poisoning is mainly acute course; however, there is interest in evaluating the effects being triggered by chronic consumption. The lack of studies on animals which are continuously exposed to phycotoxins and the scarce availability of certified reference materials have led to difficulties in evaluating risk, developing analytical methodologies and regulating these substances.

Studying toxins produced by algae has classically been approached according to the type of poisoning which they have caused. Four groups of toxins can thus be distinguished, causing paralytic shellfish poisoning, diarrhoeic shellfish poisoning, amnesic shellfish

poisoning and neurotoxic shellfish poisoning (FAO, 2004). Another group of phycotoxins of interest due to their accumulation in sea fish are the ciguatoxins causing ciguatera fish poisoning.

6.2.1 Paralytic shellfish poisoning

The toxins responsible for this poisoning are mainly produced by algae from the genera *Alexandrium, Gymnodinium* and *Pyrodinium*. Chemically, they correspond to tetrahydropurin molecules, saxitoxin (STX) having the most importance. PSP incidence and geographical distribution has increased since the 1970s; this poisoning was initially confined to temperate waters in Europe, North America and Japan, but is now considered to be worldwide problem (FAO, 2004).

STX becomes rapidly absorbed through the gastrointestinal tract and equally has rapid distribution, metabolism and excretion. STX selectively blocks (and with high affinity) sodium-dependent channels present in nerves, skeletal muscular fibres and most cardiac muscular fibres, thereby reducing or eliminating the action of propagation potential (Etheridge, 2010).

Paralytic shellfish poisoning symptoms begin in human beings within the first 30 minutes following consumption of contaminated foodstuff and the onset of numbness and/or pins and needles around the lips, gradually extending to the face, neck, arms and legs. Headaches, nausea, lack of muscular coordination and, occasionally, temporary blindness occur. There may be paraesthesia in the arms and legs, motor inability and difficulty in talking in moderate cases and paralysis of respiratory muscles leading to death may occur in severe cases.

6.2.2 Diarrhoeic shellfish poisoning

Diarrhoeic shellfish poisoning toxins are produced by dinoflagellates from the genera *Dinophysis* and *Protoceratium*, having worldwide distribution. Okadaic acid (OA) and its analogues (dinophysis toxins , pectenotoxin and yessotoxin are included within this group. However, yessotoxin and pectenotoxin produce different toxicological effects in experimental animals. Yessotoxin is related to lesions in the cardiac muscle, liver, pancreas and cerebral neurons and pectenotoxin is clearly hepatotoxic. The effect which yessotoxin and pectenotoxin may have on human beings remains unknown (Dominguez et al., 2010).

OA was initially reported in Japan and Europe, areas in which diarrhoeic shellfish poisoning has had greater importance. OA and its analogues are potent protein phosphatase inhibitors (serine/threonine phosphatases PP1 and PP2A) which dephosphorylate molecules closely related to metabolic processes. It has been postulated that OA induces diarrhoea due to an alteration in hydric balance in the intestines via one of the two following mechanisms: stimulating the phosphorylation of proteins controlling sodium secretion in enterocytes or promoting the phosphorylation of intercell binding proteins regulating solute permeability. Diarrhoeic shellfish poisoning is characterised by diarrhoea, nausea, vomiting and, in some cases, abdominal pain which can begin within the first 3 or 12 hours after having consumed contaminated organisms. No lethal effects have been reported concerning human as having been caused by OA or its analogues.

6.2.3 Amnesic shellfish poisoning

This poisoning is also known as domoic acid (DA) poisoning since memory loss is not always present. It was described for the first time in Canada (Prince Edward Island) in 1987 when 105 people became poisoned after consuming blue mussels. There have also been several reports of poisoning involving effects on wild life, demonstrating that the toxin forms part of the food chain; the toxin responsible for this has been DA which is produced by diatoms from the genera *Pseudo-nitzschia*.

The DA mechanism of action acts on excitatory amino acid receptors (L-glutamate, L-aspartate) and/or synaptic transmission. DA activates specific excitatory amino acid L - glutamate receptors producing an excessive accumulation of calcium resulting in cell death. The kainate receptor is DA's primary target. Recent interest in DA has been centred on recognising that effects can result following chronic exposure to it at low concentrations, given the discovery that chemical route alteration leads to neurological disturbances.

Intestinal absorption is limited (5%-10% of the dose administered to experimental animals). It has high distribution in the blood compartment and scarcely penetrates the hematoencephalic barrier. There is no evidence that DA may become metabolised. Elimination occurs via the kidneys. Poisoning in humans produces gastroenteritis which may be accompanied by headache, confusion and permanent loss of short-term memory (FAO, 2004).

6.2.4 Neurotoxic shellfish poisoning

Neurotoxic shellfish poisoning, which is endemic on the Gulf of México and the eastern coast of Florida, is caused by brevetoxin (BTX) produced by the dinoflagellate *Gymnodinium breve* (synonyms: *Ptychodiscus breve, Karenia brevis*) present in red-tides. This alga has the special feature of being able to form aerosols due to wave action thereby constituting a risk of aerial exposure.

BTX are liposolube toxins consisting of around 14 different substances, leading to depolarisation opening sodium channels in cell membranes and increasing the inflow of sodium causing persistent and repetitive activation. Symptoms caused by oral exposure to BTX occur within the first 30 minutes to 3 hours after consuming contaminated organisms and include vomiting, diarrhoea, shivering, sweating, conflicting perception of temperature, hypotension, arrhythmias, numbness, paraesthesia of the lips, face and extremities, cramps, bronchoconstriction, paralysis, convulsions and coma. There have been no reports of lethality. Respiratory difficulty and irritation of the mucosa are the most common symptoms when inhalatory exposure occurs.

6.2.5 Ciguatera fish poisoning

Ciguatera fish poisoning is caused by ciguatoxin (CTX) which is produced by dinoflagellates from the genera *Gambierdiscus.* CTX becomes accumulated through the food chain, from small herbivorous fish up to large carnivorous fish. This poisoning has passed from being a problem limited to insular regions which affected local communities to being a global matter, given the worldwide consumption of seafood and international tourism. This is the most common poisoning caused by seafood and may affect up to 50,000 people annually

(FAO, 2004). CTX are liposoluble toxins having 13 to 14 rings fused in a rigid structure. CTX bind to sodium channels causing them to open during cell membranes' potential repose altering bioenergetic mechanisms. CTX acts on the same receptor as BTX does but with greater affinity (Lehane & Lewis, 2000).

Gambierdiscus toxicus, the alga specie most commonly related to CTX production, is distributed throughout the tropical region of the Pacific Ocean, western Indian Ocean and the Caribbean where CFP is endemic. Many coral fish species are involved, including herbivores and carnivores. The latter constitute the main vector for poisoning in humans, particularly *Muraenidae* (moray eels), *Lutjanidae* (snappers), *Carrangidae* (carrangs), *Scombridae* (mackerels) and *Sphyraenidae* (barracuda) (FAO, 2004).

CTX become rapidly absorbed through the intestine and are mainly excreted in the faeces via the bile. The symptoms are gastrointestinal or neurological in nature, the former include vomiting, diarrhoea, nausea and abdominal pain. The neurological symptoms which can begin later include pins and needles in the lips, hand and feet, disturbances in perception of temperature, severe pruritus and fatigue. Some patients can experience pain (muscular, articular and dental) and anxiety. There may be hypotension and bradycardia in severe cases and death may occur, though this is not very common. The neurological symptoms can persist for years in some cases; it seems that the toxin may become accumulated in fat and be released in certain circumstances or also produce an immunological response (Shoemaker et al., 2010).

Other phycotoxins of interest due to their residuality are the azaspiracida, discovered in 1998, and whose symptoms resemble those of diarrhoeic shellfish poisoning; the cyclic imins (gymnodimine, spirolids, pinnatoxins, prorocentrolide, spirocentrimine and pteriatoxin), still have no associated effects in humans, but their residuality does interfere with the analytical methodologies used for determining the presence of marine toxins and cianotoxins (nodularin and cylindrospermin) by producing hepatoxicity and inhibiting protein synthesis.

6.3 Phytotoxins

Plants have secondary metabolites with which they defend themselves from the aggression of herbivorous animals. Many of these are toxic for humans and animals, causing numerous pathologies. Given that the diverse compounds present in plants are degraded during digestion and/or the metabolism of xenobiotics in animals of livestock interest, only some manage to contaminate products of animal origin. Milk is the main subproduct which has been studied in which toxins from plants may be present; however, their presence has also been demonstrated in muscle, viscera and eggs. Regulations regarding these toxins are scarce and are mainly orientated towards their presence in botanical products having pharmacological uses; however, there is growing interest in making advances in this field. The regulating entities have shown the greatest interest in pyrrolizidine alkaloids amongst the plant toxins due to their abundance and proven toxic effects.

6.3.1 Pyrrolizidine alkaloids

Pyrrolizidine alkaloids (AP) are present in a large variety of plants and are perhaps the most widely distributed toxins. Foodstuffs or botanically-based remedies represent the most

probable risk of exposure for humans; however, food of animal origin can also contain pyrrolizidine alkaloids. It has been presumed that more than 6,000 plant species contain AP, mainly those belonging to the families *Asteraceae* or *Compositae* (genera *Senecio* and *Eupatorium*), *Boraginaceae* (genera *Heliotropium* and *Echium*) and *Fabaceae* or *Leguminosae* (genera *Crotalaria*).

APs are heterocyclic compounds which are mainly derived from four necine bases: retronecine, heliotridine, otonecine and platynecine. AP can become enzimatically hydrolised or oxidised; the resulting N-oxide is slightly toxic and may also be found in plants. AP becomes bioactivated to a toxin pyrrole through CYP450 which is electrophilic and unstable and reacts rapidly with endogenous macromolecules (particularly with DNA forming adducts). DNA adducts could be a continuous source of carbon ions originating new adducts, meaning that the total elimination of AP derivates may take months (Edgar et al., 2011).

AP levels in foodstuffs are rarely so significant that they can cause acute diseases; however, low levels and continuous exposure could lead to the presentation of chronic diseases which could hardly be attributable to the toxin in foodstuffs. Pyrroles cause thickening and occlusion of the hepatic vessels resulting in veno-occlusive disease and cirrhosis. They can also alter the pulmonary vessels, causing pulmonary hypertension and congestive cardiac failure. The IARC has classified lasiocarpine, monocrotaline and riddelliine AP within group 2B (probably carcinogenic for humans, given the pertinent evidence regarding how they are cancerigens in animals) (Edgar et al., 2011).

It has been demonstrated that 0.1% to 4% of AP in foodstuffs for lactating cows and sheep could be excreted in milk (Hoogenboom, 2011). The meat and viscera of cattle fed on AP-rich plants may contain the toxin and its derivates at levels reaching 250 mg/kg in muscle and 2,500 mg/kg in liver (Fletcher et al., 2011). AP can potentially be present in eggs when fowl are fed on AP-rich diets, thereby constituting a risk for human health (Eröksüz et al., 2008). In spite of potential contamination in milk, meat, viscera and eggs, honey is the only foodstuff of animal origin which has been shown to be naturally contaminated with AP; a large number of plants habitually used in apiaries could be a source of significant levels of the toxins.

Other alkaloids of interest due to their potential residual effect on animal subproducts are the indolizidinics (especially swainsonine) producing lysosomal storage disease and piperidine (coniin and gamma-conicein) which in acute form can produce muscular paralysis and (chronically) teratogenesis (Panter & James, 1990). These last named alkaloids seem to be responsible for coturnism, a human disease which occurs following the consumption of migrating wild European quail; it is characterised by weakness, muscular pain, paralysis of the legs, vomiting and myoglobinuria. It has been postulated that these symptoms occur due to the accumulation of coniin in birds' tissues following the consumption of *Conium maculatum* (López & Bianchini, 1999).

6.3.2 Glucosinolates

Glucosinolates, known as mustard oil glucosides as they confer the characteristic flavour on black mustard (*Brassica nigra*), are secondary metabolites produced by plants belonging to the order *Brassicales*, mainly in the family *Brassicaceae (*or *Cruciferae)*. Many plants which are

common in the human diet belong to this family (broccoli, Brussels sprouts, cabbage and cauliflower) and several genera (*Brassica*, *Crambe*, *Sinapis* and *Raphanus*) including crops used for producing vegetal oils. The plant *Camelina sativa* (false flax) has recently aroused interest due to biofuel production. When the oils are extracted, the glucosinolates remain in the seed; the resulting press cake is used in animals diet (EFSA, 2008).

The glucosinolates become hydrolysed by enzymatic action giving place to isothiocyanates, thiocyanates, oxazolidinethiones and nitriles. The thiocyanates interfere with iodine capture and the oxazolidinethiones with thyroid (T_3 and T_4) hormone synthesis, leading to hypothyroidism and thyroid gland enlargement. Consequently, metabolism in all tissues, including the reproductive organs, may become affected.

It has been shown that high glucosinolate consumption in lactating cows reduces iodine levels and increases thiocyanates in milk, the liver and the kidneys. The residues in milk account for around 0.1% of the dose received by animals; the residues in muscles and viscera are even lower. Residuality has also been found in eggs after rapeseed has been administered to egg-laying birds; these also acquire a disagreeable flavour (EFSA, 2008).

6.3.3 Ptaquiloside

Bracken fern (genus *Pteridium)*, considered one of the five most abundant plants in the world, contains a norsesquiterpene glucoside called ptaquiloside. It has been proved that ptaquiloside may cause tumours in the urinary bladder, mammary glands, intestine and other organs in laboratory rodents. It causes degeneration of the retina in sheep and causes urinary bladder cancer known as bovine enzootic haematuria in cattle. There is epidemiological evidence relating the consumption of bracken fern in humans (Japanese population) suffering from esophagic and gastric cancer, possibly caused by ptaquiloside. Around 8.6% of the ptaquiloside present in *P. aquilinum*, consumed by lactating cows, is excreted in milk, thereby making contaminated raw milk a risk for human health (Alonso-Amelot et al., 1996).

6.3.4 Tremetol

This is a liposoluble compound mixture of terpene, sterols, tremetone, hydroxytremetone and dehydrotremetone; the last three are present in a ketonic fraction. It is present in the perennial plants white snakeroot (*Ageratina altissima*, previously called *Eupatorium rugosum*) and rayless goldenrod (*Haploppapus heterophyllus*). This poisoning reached an epidemic proportion during the 18th and 19th centuries in the USA (Indiana, Illinois and Ohio), producing high mortality, and its aetiological agent was only discovered in 1910. Cows accumulate the toxin in fat and excrete it in milk. The toxin becomes diluted in milk reception tanks, making such poisoning not very common in modern milk production systems. Tremetol produces acidosis, hyperglycaemia and ketonaemia, as a consequence in the Krebs cycle inhibitor. The symptoms of poisoning are anorexia, listlessness, weakness, stiffness of muscles, vomiting, constipation and coma. Marked acidosis and ketosis may lead to death (Lewis & Elvin-Lewis, 2003).

7. Conclusions

Interest in diseases caused by food has mainly been orientated towards the acute presentation principally produced by microbiological agents; however, consuming food

contaminated by chemical substances could lead to chronic exposure leading to the presentation of diseases lacking an apparent cause and being difficult to diagnose. Foods of animal origin presuppose the risk of contamination, whether from drugs and growth promoters used for optimising livestock production systems, or with biological toxins present in food ingested by animals. It is thus necessary to control these substances in foods, thereby supposing technological and institutional efforts.

Sanitary authorities must thus promulgate and ensure compliance with standards and guidelines concerning the production of harmless foodstuffs. Achieving such objective represents a great challenge for underdeveloped and developing countries due to institutional difficulties and the limited availability of equipment and qualified personnel. All nations must make it a priority to try to ensure the safe consumption of foodstuffs by their populations, exercising strict sanitary control aimed to avoid problems of health in the population and preventing the appearance of new problems affecting the development of the agro-food industry and global trade in foodstuffs.

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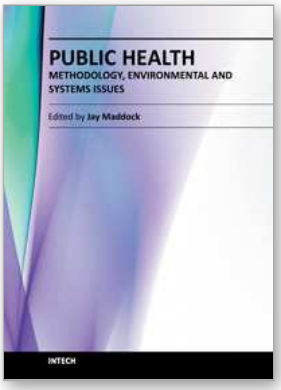

Public Health - Methodology, Environmental and Systems Issues
Edited by Prof. Jay Maddock

ISBN 978-953-51-0641-8
Hard cover, 432 pages
Publisher InTech
Published online 30, May, 2012
Published in print edition May, 2012

Public health can be thought of as a series of complex systems. Many things that individual living in high income countries take for granted like the control of infectious disease, clean, potable water, low infant mortality rates require a high functioning systems comprised of numerous actors, locations and interactions to work. Many people only notice public health when that system fails. This book explores several systems in public health including aspects of the food system, health care system and emerging issues including waste minimization in nanosilver. Several chapters address global health concerns including non-communicable disease prevention, poverty and health-longevity medicine. The book also presents several novel methodologies for better modeling and assessment of essential public health issues.

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